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cc

Sterne Kessler Goldstein Fox
ATTORNEYS AT LAW

December 1, 2004

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Certificate
DEC 0 7 2004
of Correction

Commissioner for Patents
PO Box 1450
Attn: Certificate of Correction Branch
Alexandria, VA 22313-1450

Re: U.S. Utility Patent No. 6,735,530 B1; Issue Date: May 11, 2004
(from Application No. 09/183,267; Filed: October 30, 1998)
For: **Computational Protein Probing to Identify Binding Sites**
Inventor: Frank GUARNIERI
Our Ref: 1866.0010001/PEG/CMB

Sir:

Transmitted herewith for appropriate action are the following documents:

1. Fee Transmittal (PTO/SB/17);

2. Request for Certificate of Correction Under 37 C.F.R. § 1.322 and 1.323 (with Exhibits 1-3);

3. Certificate of Correction (3 pages) (PTO/SB/44);

4. PTO-2038 Credit Card Payment Form for $100.00 to cover Certificate of Correction; and

5. Return postcard.

It is respectfully requested that the attached postcard be stamped with the date of filing of these documents, and that it be returned to our courier. In the event that extensions of time are necessary to prevent abandonment of this patent application, then such extensions of time are hereby petitioned.

DEC 0 7 2004

The U.S. Patent and Trademark Office is hereby authorized to charge any fee deficiency, or credit any overpayment, to our Deposit Account No. 19-0036.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

for Patrick E. Garrett Reg. No. 47,438
Attorney for Patentee
Registration No. 39,987

PEG/slw
Enclosures

339610_1.DOC

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent of: Frank GUARNIERI	Confirmation No.: 2934
Patent. No.: 6,735,530 B1	Art Unit: 1631
Issued: May 11, 2004	Examiner: Borin, Michael L.
For: **Computational Protein Probing to Identify Binding Sites**	Atty. Docket: 1866.0010001/PEG/CMB

Request for Certificate of Correction Under 37 C.F.R. § 1.322 and 1.323

Attn: Certificate of Correction Branch

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

It is hereby requested that a Certificate of Correction under 37 C.F.R. §§ 1.322 and 1.323 be issued for the above-captioned United States Patent. This Certificate of Correction is being requested due to mistakes which appear in the printed patent. These mistakes were made by both the U.S. Patent and Trademark Office and by Applicant. The mistakes made by Applicant are of a clerical or typographical nature, or of a minor character. Patentee submits that correction of these errors does not introduce new matter.

Specifically, the printed patent contains the errors listed below for which a Certificate of Correction is respectfully requested. These grouping of these errors is based on where support for each error can be found.

Applicant Errors

The following corrections are requested to correct minor errors made by Applicant:

In column 3, line 55, "Boactive" should be replaced with --Bioactive--. This change is to correct a spelling error.

In column 4, line 47, ""ORFs" should be replaced with --"ORFs"--. This change is to correct an accidental omission of a quotation mark.

In column 11, line 38, "$P_N^{cav}N$" should be replaced with --$P_N^{cav}V$--. This change is to correct an incorrectly typed equation.

PTO Errors - Based On November 24, 2003 IDS

In section (56), the references that were cited in the Information Disclosure Statement of November 14, 2003 should be listed. A copy of the Examiner-initialed form PTO-1449 is enclosed as Exhibit 1, showing that the references were considered by the Examiner.

PTO Errors - Based On Original Application

Support for the following PTO errors can be found in the original application, where noted, which is enclosed as Exhibit 2.

In column 8, line 19, "prolyl-pisopropylanilide" should be replaced with --prolyl-*p*-isopropylanilide--. Support for this correction can be found on page 11 of the original application.

In column 10, line 47 (equation 5), "+l*N*>" should be replaced with --+ln<*N*>--. Support for this correction can be found on page 15 of the original application.

In column 10, line 66, "IIV" should be replaced with --1/V--. Support for this correction can be found on page 15 of the original application.

In column 11, line 32 (equation 8), "$P_N^{cav}N$" should be replaced with --$P_N^{cav}V$--. Support for this correction can be found on page 16 of the original application.

In column 14, line 54, "calorimetric" should be replaced with --colorimetric--. Support for this correction can be found on page 21 of the original application.

PTO Errors - Based On September 9, 2003 Amendment

Support for the following PTO errors can be found in the Amendment of September 9, 2003, enclosed as Exhibit 3.

In column 9, line 13, "*Strict.*" should be replaced with –*Struct.*--.

In column 9, line 19, "n-tert-butoxycarbonyl" should be replaced with --n-(tert-butoxycarbonyl--.

In column 13, line 23, "and fold" should be replaced with --and 5 fold--.

In column 13, line 42, "DNA:" should be replaced with --DNA.--.

In column 15, line 10, "represculation" should be replaced with --representation--.

In column 15, line 13, "R=" should be replaced with --B=--.

In column 15, line 14, "μ' the" should be replaced with --μ' is the--.

In column 15, line 29, "is organic" should be replaced with --is an organic--.

In column 15, line 34, "outputted step" should be replaced with --outputted in step--.

In column 15, line 40, "steps" should be replaced with --step--.

In column 15, line 46, "of recording" should be replaced with --according--.

In column 15, line 46, the "a" after "wherein" should be deleted.

In column 15, line 50, "fragment" should be replaced with --fragments--.

In column 15, line 53, "lower" should be replaced with --lowest--.

In column 15, line 54, "performed," should be replaced with --performed;--.

In column 15, line 63, "benzeue" should be replaced with --benzene--.

In column 15, line 64, "formadehyde" should be replaced with --formaldehyde--.

In column 16, line 10, "further" should be deleted.

In column 16, line 16, "μ' the" should be replaced with --μ' is the--.

In column 16, line 17, "constant T" should be replaced with --constant, T--.

In column 16, line 17, "temperature;" should be replaced with --temperature,".

In column 16, line 27, "leaser" should be replaced with --lesser--.

In column 16, line 28, "molecule molecular" should be replaced with --molecule or molecular--.

In column 16, line 60, "list unrejected" should be replaced with --list of unrejected--.

Remarks

The above-noted corrections do not involve such changes in the patent as would constitute new matter or would require reexamination.

A completed Form PTO/SB/44 accompanies this request, with the above-noted corrections printed thereon. Accordingly, a Certificate of Correction is believed proper and issuance thereof is respectfully requested.

This request is accompanied by payment of the fee set forth in 37 C.F.R. § 1.20(a). The Commissioner is hereby authorized to charge any fee deficiency, or credit any overpayment, to our Deposit Account No. 19-0036.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

for Patrick E. Garrett
Attorney for Patentee
Registration No. 39,987

Reg. No. 47,438

Date: December 1, 2004

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324942_1.DOC

PTO/SB/17 (10-04v2)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

FEE TRANSMITTAL for FY 2005

Effective 10/01/2004. Patent fees are subject to annual revision.

☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 100.00

Complete if Known	
Application Number	09/183,267
Filing Date	October 30, 1998
First Named Inventor	Frank GUARNIERI
Examiner Name	Borin, Michael L.
Art Unit	1631
Attorney Docket No.	1866.0010001/PEG/CMB

METHOD OF PAYMENT *(check all that apply)*

☐ Check ☒ Credit card ☐ Money Order ☒ Other ☐ None

**Charge any deficiencies or credit any overpayments in the fees to Deposit Acct. No. 19-0036.

☐ Deposit Account:

Deposit Account Number: 19-0036

Deposit Account Name: Sterne, Kessler, Goldstein & Fox P.L.L.C.

The Director is authorized to: *(check all that apply)*

☐ Charge fee(s) indicated below ☐ Credit any overpayments

☐ Charge any additional fee(s) or any underpayment of fee(s)

☐ Charge fee(s) indicated below, **except for the filing fee** to the above-identified deposit account.

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	790	2001	395	Utility filing fee	
1002	350	2002	175	Design filing fee	
1003	550	2003	275	Plant filing fee	
1004	790	2004	395	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	

SUBTOTAL (1) ($) 0

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

		Extra Claims		Fee from below	Fee Paid
Total Claims	10 -20** =	0	X	18.00	= 0
Independent Claims	2 -3** =	0	X	88.00	= 0
Multiple Dependent				300.00	= 0

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	88	2201	44	Independent claims in excess of 3
1203	300	2203	150	Multiple dependent claim, if not paid
1204	88	2204	44	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 0

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	430	2252	215	Extension for reply within second month	
1253	980	2253	490	Extension for reply within third month	
1254	1,530	2254	765	Extension for reply within fourth month	
1255	2,080	2255	1,040	Extension for reply within fifth month	
1401	340	2401	170	Notice of Appeal	
1402	340	2402	170	Filing a brief in support of an appeal	
1403	300	2403	150	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,370	2453	685	Petition to revive - unintentional	
1501	1,370	2501	685	Utility issue fee (or reissue)	
1502	490	2502	245	Design issue fee	
1503	660	2503	330	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	790	2809	395	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	790	2810	395	For each additional invention to be examined (37 CFR 1.129(b))	
1801	790	2801	395	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) Certificate of Correction 100.00

*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) ($) 100.00

SUBMITTED BY

(Complete *(if applicable)*)

Name (Print/Type)	Patrick E. Garrett	Registration No. (Attorney/Agent)	39,987	Telephone	(202) 371-2600
Signature	for Reg. No. 47,438			Date	Dec. 1, 2004

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

FORM PTO-1449 FIRST SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT	ATTY. DOCKET NO. 1866.0010001	APPLICATION NO. 09/183,267
	APPLICANT Frank Guarnieri	
	FILING DATE October 30, 1998	GROUP 1631

U.S. PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUB-CLASS	FILING DATE
	AA						
	AB						
	AC						
	AD						
	AE						
	AF						
	AG						
	AH						
	AI						
	AJ						
	AK						

FOREIGN PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUB-CLASS	TRANSLATION
	AL						Yes No
	AM						Yes No
	AN						Yes No
	AO						Yes No
	AP						Yes No

OTHER (Including Author, Title, Date, Pertinent Pages, etc.)

MM	AR	1	Mezei, M., and Beveridge, D.L., "Structural Chemistry of Bimolecular Hydration via Computer Simulation: The Proximity Criterion," in *Methods in Enzymology*, Parker, ed., Academic Press, NY, pp. 21-47(1986).
MM	AS	1	Mezei, M., "Modified Proximity Criteria for the Analysis of the Solvation of a Polyfunctional Solute," *Mol. Simul.* 1:327-332, Gordon and Breach Science Publishers S.A. (1988).
MM	AT	1	Mezei, M., "Grand-canonical ensemble Monte Carlo study of dense liquid Lennard-Jones, soft spheres and water," *Mol. Phys.* 61:565-582, Taylor and Francis (1994).

EXAMINER _____ DATE CONSIDERED 2/2005

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

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SKGF Rev. 1/95

COPY

FORM PTO-1449	ATTY. DOCKET NO. 1866.0010001	APPLICATION NO. 09/183,267
FIRST SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT	APPLICANT Frank Guarnieri	
	FILING DATE October 30, 1998	GROUP 1631

U.S. PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUB-CLASS	FILING DATE
	AA						
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	AG						
	AH						
	AI						
	AJ						
	AK						

FOREIGN PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUB-CLASS	TRANSLATION
	AL						Yes No
	AM						Yes No
	AN						Yes No
	AO						Yes No
	AP						Yes No

OTHER (Including Author, Title, Date, Pertinent Pages, etc.)

MM	AR	2	Mehrotra, P.K., and Beveridge, D.L., "Structural Analysis of Molecular Solutions Based on Quasi-Component Distribution Functions," *J. Am. Chem. Soc. 102*:4287, American CHemical Society (1980).
MM	AS	2	Metropolis, N., *et al.*, "Equation of State Calculations by Fast Computing Machines," *J. Chem. Phys. 21*:1087-1092, American Institute of Physics (1953).
MM	AT	2	Resat, H., and Mezei, M., "Grand canonical Monte Carlo Simulation of Water Positions in Crystal Hydrates," *J. Am. Chem. Soc. 116*:7451-7452, American Chemical Society (1994).

EXAMINER MM DATE CONSIDERED 12/2005

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

Computational Protein Probing to Identify Binding Sites

The present application claims the priority of US Provisional Application No. 60/101,521, filed September 23, 1998.

The present invention relates to methods of identifying binding sites on proteins, methods for identifying classes of compounds suitable for binding a protein, and methods of conducting experiments to identify compounds that interact with a protein to affect a biological process.

Determinations of protein structures have to date been conducted by isolating crystals of the protein of interest, and analyzing structure by X-ray crystallography. Typically, the protein has been co-crystallized with heavy metal component, or subjected to multiple co-crystallizations, with the heavy metal providing a reference for solving the crystallographic data.

With a determination of the structure of a protein, or the structure of another macromolecule having significant tertiary structure, such as a DNA or RNA, workers often seek to identify the binding sites that are or may be of significance to a biological process, such as an enzyme active site or a site for interacting with another macromolecule or with itself. Computational efforts have been focused on efforts to sample the surface of a molecule to find good fits with known binding agents. These methods have had modest success, and are dependent on knowledge of (a) the structure of good binding agents and, often, (b) the function of the protein. A more traditional approach has sought to co-crystallize binding substances with the macromolecule to identify binding sites. With the binding site identified, educated guesses can be made as to new molecules that could bind the site. These educated guesses can guide synthetic methods, including combinatorial chemistry methods, to make and test new molecules. When such prospective binding agents prove effective binding agents, and possibly are also found effective in an appropriate biological model, the structural correlations drawn from the results can be tied to information about the binding site to make still further inferences about the structure important to a biological function. This co-crystallization approach depends on an initial knowledge of active agents, and is experimentally difficult and time consuming.

The present inventor has found a method of identifying, from a three-dimensional structural solution of a macromolecule, the binding sites for molecules. The structural solution used as the basis for the method can be derived from crystallography, spectroscopic analyses such as NMR, computational derivations, or any other method of determining the structure of a macromolecule. The method does not require or typically use information on the function of the macromolecule, as the method avoids subjective biases and instead depends purely on physical parameters. Further, the method can be refined further to narrow the possible choices of binding sites and identify the functionalities, i.e., organic fragments or "ORFs," that effectively interact with the binding site(s). The data obtained for ORFs further identifies the orientations of the functionalities useful in a candidate binding agent, thereby providing a tool for searching chemical databases to identify candidate binding agents. Where the methods described herein identify more than one potential binding site, the data generated through these methods can be used to energetically rank the binding sites, and thereby quantitatively determine which site has the potential to more strongly bind molecules.

The computational method described here generates maps of binding site preferences that are nearly identical with maps produced by compiling data generated by traditional methods, but with one important difference – the experimentally produced data took many years to produce while the data produced as described herein can be produced in no more than a few weeks. The invention provides an important development in unbiased simulation methods for predicting the character of agents that bind to biological macromolecules to affect the function of the macromolecules.

Summary of the Invention

In one embodiment, provided is a method of identifying binding sites on a macromolecule comprising: **(a)** for at least one organic fragment (ORF), conducting, at separate values of parameter *B*, two or more simulated annealing of chemical potential calculations using the ORF as the inserted solvent; and **(b)** comparing converged solutions from step (a) to identify first locations at which the relevant ORF is strongly bound, thereby identifying candidate sites for binding ligand molecules. In one preferred aspect, the method further comprises: **(c)** identifying clusters of sites that strongly bind an ORF. In another preferred aspect, the method further comprises: **(d)** conducting steps

(a) and (b) for each of two or more ORFs and identifying clusters where two or more distinct ORFs bind. Preferably, a cluster that binds three or more distinct ORFs is identified. The method can identify further functionalities that contribute to the binding of bioactive agents by reducing the binding stringency in the vicinity of a cluster to further identify elements that would contribute to the binding of a bioactive agent.

In another preferred aspect, the method further comprises: (e) conducting, at separate values a measure of chemical potential, two or more simulated annealing of chemical potential calculations using water as the inserted solvent; (f) comparing converged solutions from step (c) to identify locations at which water is strongly bound, thereby identifying locations on the protein which are not candidate sites for binding ligand molecules; and (g) identifying first locations that are not water locations.

In still another preferred aspect, the simulated annealing of chemical potential calculations comprise multiple steps of sampling, and wherein in a number of steps of the sampling the ORFs position is changed by a small amount and the resulting new position is accepted or rejected based on the change in energy as a result of the change attempted.

Further provided is a method of identifying the chemical characteristics of compounds that bind a macromolecule comprising examining the functionalities and relative orientations of the ORFs found in a cluster pursuant to the binding site identifying method outlined above.

Also provided is a method of conducting combinatorial chemistry to identify compounds that interact with a macromolecule comprising: (a) identifying classes of reactants that are modeled by the functionalities of the ORFs found in a cluster pursuant to the binding site identifying method of macromolecule; (b) designing a combinatorial synthetic protocol that calls for two or more synthetic procedures that react reagents of at least two of the classes identified in step (a); and (c) conducting the combinatorial synthetic protocol to create candidate binding molecules.

Further provided is a method of conducting a bioactive agent discovery process comprising: (a) from a group of established combinatorial synthetic protocols or a collections of chemical compounds or pools of chemical compounds, identifying those members of the group that provide a high density of compounds that meet for a macromolecule selection criteria identified from the binding site identifying method of macromolecule; and (b) conducting binding or functional assays to identify compounds

obtained from the identified collections or protocols which bind or affect the function of the macromolecule.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A illustrates a solved crystal structure, while **Figure 1B** displays the structure with a grid is imposed.

Figures 2A-2D display the method of the invention applied to the crystallographic solution of elastase, the method can be exemplified using methanol as the ORF.

Figures 3A and **3B** show the combined results for several ORFs bound to elastase after simulations at relatively low *B* values, with the results in **Figure 3B** filtered to identify clusters of these bound ORFs.

Figure 3C shows the two clusters of **Figure 3B** which remain after excluding strong water binding sites, and **Figure 3D** shows the one cluster that remains after extending the analysis to another ORF; **Figure 3E** shows the analysis extended to still a further ORF.

The panels of **Figure 3F** compare the simulation results to a co-crystallography result.

Illustrated in **Figure 4A** are the amide binding sites extracted from the data of six co-crystallization experiments with elastase and known ligands; and illustrated in **Figure 4B** is a cluster of the highest affinity amide binding sites determined by the simulation method of the invention.

Illustrated in **Figure 4C** are the amide ORFs of **Figure 4B** plus amides which are in the vicinity of the cluster but which appear in the simulation at second highest affinity binding values.

In **Figures 5A** and **5B**, solutions obtained with co-crystals of elastase inhibitors are compared with data obtained by the methods herein described.

Figures 6A and **6B** show the surfaces of elastase involved in binding ligands as indicated by the crystallographic data, **Figure 6A**, and as indicated by the solutions obtained the method described herein, **Figure 6B**.

Figure 7 shows a schematic illustration of the type of titrations for water binding to a macromolecule that can be used to help identify a level of relatively strong water binding.

GLOSSARY

The following terms shall have, for the purposes of this application, the respective meaning set forth below.

- **"Boactive agent"** refers to a substance such as a chemical that can act on a cell, virus, organ or organism, including but not limited to drugs (i.e. pharmaceuticals) to create a change in the functioning of the cell, virus, organ or organism. In a preferred embodiment of the invention, the method of identifying bioactive agents of the invention is applied to organic molecules having molecular weight of about 600 or less or to polymeric species such as peptides, proteins, nucleic acids, proteoglycans and the like. A bioactive agent can be a medicament, i.e. a substance used in therapy of an animal, preferably a human.
- **"Cluster of free grid points"** refers to free grid points that are within a "cluster" in that, relative to a given ORF, there is a sufficient number of nearby or adjacent free grid points to allow a reasonable probability that the ORF could be inserted at the cluster. Thus, the cluster of free grid points for H_2O must be defined to identify all volumes at the surface or interior of a macromolecule that could accommodate H_2O – though the selection criteria should err to identifying some volumes that do not accommodate H_2O, as needed to assure that all appropriate volumes are sampled in the simulation process. A cluster of free grid points is defined differently depending on the size of the ORFs (e.g., compare H_2O and benzene) and the spacing of the grid.
- A **"cluster of ORF binding sites"** typically refers to a pattern of closely located or superimposed sites that bind ORFs with sufficient affinity to merit further consideration.
- **"Collection of chemical compounds"** refers to any collection of compounds collected or organized with the intention that they can be examined to identify bioactive agents (e.g., having a biological activity measured directly or through a surrogate for biological activity such as binding to a macromolecule or interfering with a function of a macromolecule). The collection can be prepared from a collection of simpler molecules (which can be bound to a support) by a chemical scheme designed to generate a diversity

of chemicals. Collections of this latter type are often referred to as "combinatorial libraries."

• **"Free grid points"** refers to grid points (which are discussed below) which are, for a given accepted definition of atomic radius, "free" in that they do not fall within the atomic radii of the mapped atoms of the relevant macromolecule.

• **"Macromolecule"** refers to a molecule or collection of molecules which has a time-averaged tertiary structure. Thus, while the term typically refers to proteins, ribonucleic acids, structures formed of both nucleic acid and protein, carbohydrates, structures formed of two or more of the aforementioned, and the like, it can also refer to structures formed with other molecules including lipids. Macromolecules are used in the method described herein with reference to maps of their tertiary structure. Such maps are typically generated by X-ray diffraction studies, which have generated maps for thousands of macromolecules. However, maps can be produced by other methods such as computational methods or computational methods supplemented by other data such as NMR data. While computational methods have been difficult to apply, recent studies appear to have achieved some successes.

• **Organic fragments or "ORFs** are molecules or molecular fragments that can be used to model one or more modes of interaction with a macromolecule, such as the interactions of carbonyls, hydroxyls, amides, hydrocarbons, and the like.

• **Water locations** are locations at which water is strongly bound, meaning, in one embodiment, for example locations where the simulation indicates water remains bound when the simulation is run at values of B that are equal to or less than the B value for the transition point indicating those water molecules that are strongly influenced by the macromolecule. Illustrated in **Figure 7** is a conceptualization of the titration of simulated bound water molecules with decreasing values of B, a parameter described further below. A transition point indicates water molecules that are strongly influenced by the macromolecule. A B value less than or equal to that at the transition point can be designated as defining water binding of sufficient strength to render competitive binding by another molecule unlikely, as illustrated by point **SB** in the illustration. Typically, for a water soluble protein, this point **SB** is selected so that about 100 to about 50 water molecules remain bound for a 50 kd protein.

DETAILED DESCRIPTION OF THE INVENTION

The simulation process of the present invention works by artificially inserting a given ORF at an unbiased sampling of all the sites on or within a macromolecule structure where such ORF can, as a practical matter, reside. These sites can be termed the "sampling sites." Typically, a schedule of simulations for each of a number of ORFs are run, with each simulation run at a separate value of a parameter *B*, which is related to the excess chemical potential. The schedule provides for simulations conducted at each of a number of *B* values, typically ranging from 10 to about -15. In each simulation at a given value of *B*, the simulation assesses at each step of the simulation whether the insertion of the ORF at a given site shall be accepted or rejected, with the assessment based on a grand canonical ensemble probability density function. At each step of the simulation, the algorithm models the insertion of the ORF at the site. A forced bias canonical probability density function is used to translate and rotate the ORF in small steps (e.g., ±0.2Å, ±30°) to identify an energy minimized insertion given the simulation parameters in place at the time of the simulation step. The probability of the insertion is then determined from the grand canonical ensemble probability density function, and the ORF can be represented as resident at the site by a random number generating protocol weighed to the probability value. Alternative methods for choosing to make this representation, such as applying cutoff values for when to represent the insertion or not, can also be applied, but are less favored. Typically, following a successful insertion, the subsequent deletion attempts at the site are with the previously identified translated and rotated ORF, and this translated and rotated ORF is used until a deletion attempt succeeds. The simulation is typically conducted for a large number of steps, such as 2×10^6 steps, with the majority of the steps, e.g., 1.5×10^6 required to "equilibrate" the simulation so that the number of accepted insertions is equal to the number of deletions on average.

By taking a large number of unbiased samplings at each sample site over the latter course of the simulation, such as after every 200 steps of iterations after equilibrium is achieved, an occupation probability of the ORF residing at that sample site at the given value of *B* can be assessed. The occupancy as an overall result of the method can then be determined based on this probability, for example with a random number protocol

making the representation based on its probability. The degree to which the ORF is translated or rotated can also be represented based on the probability of such translations and rotations.

For each ORF, simulations are run at each of a number of values of a measure of excess chemical potential, such as *B*. Thus, as this value lowers, the retention of an ORF at a given sampling site is an indication of high relative binding affinity.

The sampling sites are typically arrived at by creating a grid as illustrated in Figure 1. Figure 1 illustrates a solved crystal structure (Figure 1A) on which a grid is imposed (Figure 1B). For example, the grid can have about ½ Å to about 1 Å spacing, with the grid intersection points defining the candidates for sampling sites. The spacing of the grid is preferably selected to be less than the smallest cross-section of the ORF. The spacing is typically selected to be small enough in relation to the size of the ORF so that the probability that free volumes that could define free grid point clusters have sufficient free grid points to allow useful sampling as described below. Such relatively small spacing minimizes the chance that the selection of how to orient the grid will bias the algorithm against identifying certain ORF binding preferences. The sampling sites are selected from sites that are unoccupied by the macromolecule (Figure 1B). A final elimination of "grid bias" is achieved by varying the test insertion points away from strict initial insertion at grid points, as described below.

The sampling sites are limited to those sites having enough adjacent volume free of the macromolecule to allow the ORF to be inserted. For example, the sampling sites can be limited to grid points within an open area of at least about 2Å x 2Å x 2Å (= 8Å^3 or 0.008nm^3) or about 2.5Å x 2.5Å x 2.5Å (= 15.6Å^3 or 0.0156nm^3) or, for water, about 2.2Å x 2.2Å x 2.8Å. The grid points can be selected for those free grid points that are within a cluster of free grid points, such as, for example, a cluster of 3, 4, 5, 6, 7, 8 or more free grid points, depending on the size of the ORF and the spacings of the grid.

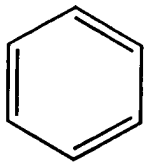
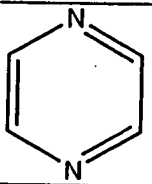
In one preferred embodiment, the ORF is not necessarily initially inserted exactly at the grid points, but instead at a random sampling of insertion points within a short distance of the grid points, such as points within a sphere shape centered at the grid point and having a diameter of about some percentage, such as 10%, of the grid spacing, or within a box shape centered at the grip point having width, length and height of about such a percentage of the grid distance. As discussed above, this "wobble" in the initial

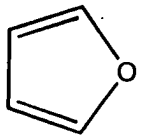
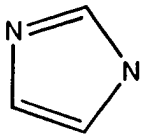
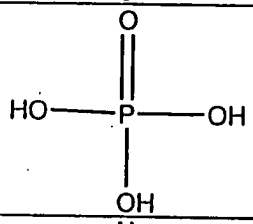
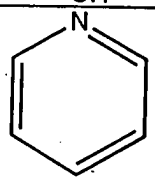
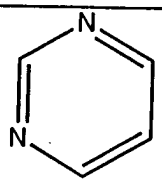
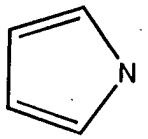
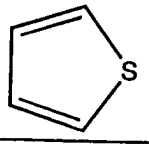
insertion point helps eliminate grid bias where the placement of the grid happens to reduce the chance that a given open volume will be efficiently sampled.

Using the crystallographic solution of elastase, in particular, the pig pancrease elastase structural solution of G.A. Petsko of Brandeis University, the method can be exemplified using methanol as the ORF. **Figure 2A** shows the final solution using a relatively high *B* value, e.g., *B* = 10. **Figure 2B** shows the final solution using an intermediate value, e.g., *B* = 6 or 7. **Figure 2C** shows the final solution using a lower intermediate value, e.g., *B* = 0 or -2 or -4. **Figure 2D** shows the final solution using a restrictive value, such as *B* = −14. As illustrated, with lower values of *B* less and less methanol molecules remain bound. These remaining methanol fragments indicate those that bind with relatively high affinity.

The next step of the process is to conduct simulations with additional ORFs and identify clusters of relatively high affinity ORF binding sites. Thus, for example, again using elastase, simulations can be conducted to determine binding for ORFs for ammonia, methanol, ketone and amide. Combined results at relatively low *B* values are illustrated in **Figure 3A**. Clusters of ORF binding sites are identified in **Figure 3B**. The method of the present invention seeks to identify clusters of ORF binding sites, where the clusters can be made up solely of one type of ORF. Preferably, however, the cluster will include binding sites for 2, 3, 4, 5, 6, 7 or more distinct ORFs.

Examples of useful ORFs include:

Name	Structure
Acetone	$CH_3(C{=}O)CH_3$
Aldehyde	$H(C{=}O)\text{-}CH_3$
Amide	$H(C{=}O)NH_2$
Ammonia	NH_3
Benzene	
Carboxylic Acid	CH_3COOH
1,4-Diazine	

Name	Structure
Ester	CH_3-O-(C=O)-CH_3
Ether	CH_3-O-CH_3
Formaldehyde	H_2C=O
Furan	
Imidazole	
Methane	CH_4
Methanol	CH_3OH
Phospho-Acid	
Pyridine	
Pyrimidine	
Pyrrole	
Thiol	CH_3SH
Thiophene	

Preferably, the ORFs selected are representative of chemical features that have proven useful in the design of pharmaceuticals or other bioactive chemicals.

Thus, in a first mode of analysis, an important part of the process is to run the simulations with several ORFs, identifying clusters of sites that bind multiple ORFs with

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No.: 2934
Frank GUARNIERI	Art Unit: 1631
Appl. No. 09/183,267	Examiner: Borin, Michael L.
Filed: October 30, 1998	Atty. Docket: 1866.0010001/JMC/PEG (*formerly* SAR-12902)
For: **Computational Protein Probing to Identify Binding Sites**	

Amendment And Reply Under 37 C.F.R. § 1.111

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

In reply to the Office Action dated March 12, 2003, Applicant submits the following Amendment and Remarks. This Amendment is provided in the format prescribed in the final rule, Changes to Implement Electronic Maintenance of Official Patent Application Records, 68 Fed. Reg. 38611, 38628 (June 30, 2003) (to be codified at 37 C.F.R. pt. 1), and in the following format:

(A) Each section begins on a separate sheet;

(B) Starting on a separate sheet, amendments to the specification by presenting replacement paragraphs marked up to show changes made;

(C) Starting on a separate sheet, a complete listing of all of the claims:

- in ascending order;

- with status identifiers; and

- with markings in the currently amended claims;

(D) Starting on a separate sheet, the Remarks.

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned for under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

Amendments

In the Specification:

On page 4, on the line following the heading "Brief Description of the Drawings," please add the following paragraph:

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Please replace the paragraph beginning on page 12, line 20 with the following paragraph:

In Figures 5A and 5B, solutions obtained with co-crystals of elastase inhibitors are compared with data obtained by the methods herein described. In Figure 5A, the solutions for six co-crystallized inhibitors are shown, with the inhibitor molecules overlaid on each other (non-space-filling representation, with the elastase segment represented by a space-filling illustration). These inhibitors are trifluoroacetyl-l-lysyl-l-prolyl-p-isopropylanilide (Mattos, C. *et al., Nat. Struct. Biol. 1*:55-58 (1994); crystal solution: PDB ID: 1ELA ~~Mattos et al., as submitted April 30, 1994~~), trifluoroacetyl-l-lysyl-l-leucyl-p-isopropylanilide (Mattos, C. *et al., Nat. Struct. Biol. 1*:55-58 (1994); crystal solution: PDB ID: 1ELB ~~Mattos et al., as submitted June 22, 1994~~), trifluoroacetyl-l-phenylalanyl-p-isopropylanilide (Mattos, C. *et al., Nat. Struct. Biol. 1*:55-58 (1994); crystal solution: PDB ID: 1ELC ~~Mattos et al., as submitted April 30, 1994~~), trifluoroacetyl-l-phenylalanyl-l-alanyl-~~p-trifluoromethylanimide~~ p-trifluoromethylanilide (Mattos, C. *et al., Biochemistry 34*:3193-203 (1995); crystal solution: PDB ID: 1ELD ~~Mattos et al., as submitted February 14, 1995~~),

trifluoroacetyl-l-valyl-l-alanyl-p-trifluoromethylanilide (Mattos, C. *et al.*, *Biochemistry 34*:3193-203 (1995); crystal solution: PDB ID: 1ELE ~~Mattos et al., as submitted February 14, 1995~~) and n-(tert-butoxycarbonyl-alanyl-alanyl)-o-(p-nitrobenzoyl) hydroxylamine (Ding, X. *et al.*, *Biochemistry 34*:7749-56 (1995); crystal solution: PDB ID: 1ELF ~~Ding et al., as submitted July 10, 1995~~). In Figure 5B, the solutions for approximately 10 ORFs, which are in their respective high affinity protein binding states are overlaid. Both methods identify a region which favors the binding of aromatic moieties. The simulation process achieves approximately 90% 3D geometric identity with the crystallography results.

Please replace the paragraph beginning on page 17, line 2 with the following paragraph:

Aspects of the simulations used in the invention can be illustrated with calculations used to determine the strength of water binding to a synthetic polynucleotide (Guarnieri, F., and Mezei, M., *J. Am. Chem. Soc. 118*:8493-8494 (1996)).[[[1]]] This illustration can be described as follows:

Please replace the paragraph beginning on page 17, line 10 with the following paragraph:

Grand canonical ensemble simulations are generally performed by placing a molecule in a periodic simulation cell, setting a parameter *B*, which is representative of free energy, in such a way as to achieve an experimentally determined density, sampling potential hydration positions around the molecule by inserting and deleting water molecules from the simulation cell using a technique such as cavity-bias (Mezei, M., *Mol. Phys. 61*:565-582 (1994); Resat, H., and Mezei, M., *J. Am. Chem. Soc. 116*:7451-7452

(1994)),[[2,3]] and accepting or rejecting the attempt based on a Metropolis Monte Carlo (Metropolis, N. *et al., J. Chem. Phys. 21*:1087-1092 (1953))[[4]] criteria using a grand canonical ensemble probability function (Tolman, R., in *The Principles of Statistical Mechanics*, Dover Press, New York (1971)).[[5]] The parameter B is related to the excess chemical potential μ' as follows: $B = \mu'/kT + ln\langle N\rangle$, where k is Boltzmann's constant, T is the absolute temperature, and $\langle N\rangle$ is the mean number of molecules of the ORF, which here is H_2O. In the method of *simulated annealing of chemical potential*, the simulation is started with a large initial B-value so that a higher percentage of water insertion attempts are accepted. This causes the simulation cell to be flooded with water molecules. After this grand canonical ensemble simulation at high excess chemical potential is equilibrated, subsequent simulations are carried out at successively lower B-values. This successive lowering of the B-values causes a gradual removal of the bulk water molecules from the simulation cell. As the chemical potential is further "annealed", a point is reached at which water molecules do not readily leave the cell, thereby identifying those water molecules that are strongly influenced by the DNA, the so-called "bound water molecules". As the excess chemical potential is again lowered, ultimately some of these bound waters start to leave the cell. Since chemical potential is a free energy, this *simulated annealing of chemical potential* yields a numerical estimate of the differential free energy of binding of the different bound water molecules. It must be emphasized that our utilization of the term "annealing" applies strictly to the value of the chemical potential and that the temperature is kept constant at, for example, 298 K in all the simulations. For all simulations the DNA was held fixed, water molecules were added and deleted throughout all parts of the cell,

relatively high affinity. These clusters are strong candidate sites for ligand binding sites. Moreover, the relative positioning of the ORFs is instructive of the features of good binding agents. For example, at the binding site identified on elastase by the methods described below, a cluster having two benzene rings with an amide interposed between them models some of the strongest elastase inhibitors derived from an extensive research program, which inhibitors have a sulfonamide in place of the carbon-based amide of the simulation. See, Tables XXIII and XXV of Edwards et al., "Synthetic Inhibitors of Elastase," *Medicinal Research Reviews* 14:127-194, 1994.

In some implementations of the invention, clusters of ORF binding sites alone will identify, or substantially narrow the range of choices for, the sites at which ligands interact with a given protein. However, in some embodiments of the invention, the sites that bind water strongly are identified, and the clusters that intersect with strong water binding sites are discounted. Thus, in the elastase example, the candidate ligand binding sites of **Figure 3B** are narrowed by excluding water binding sites, as illustrated in **Figure 3C**. If the analysis is extended to five ORFs as illustrated in **Figure 3D**, a single candidate site remains. **Figure 3E** shows a slightly different perspective of the same site illustrated in **Figure 3D**, with the analysis extended to six ORFs. **Figure 3F** shows how well the candidate site (left panel) matches up with the structure of a co-crystal containing the ligand trifluoroacetyl-lysyl-prolyl-*p*-isopropylanilide.

Accordingly, in a second mode of analysis, an optional step in the process is to narrow the choices for ligand binding sites by excluding ORF clusters that intersect with relatively strong water binding sites.

It should be noted that clusters of ORFs are typically identified at relatively low *B* values, thereby helping to identify prospective binding sites for ligands. However, further information about prospective binding sites can be gleaned by looking, in the vicinity of a prospective binding site, at more weakly binding ORFs. This information value flows from the prospect of more weakly binding ORFs modeling a ligand interaction which, while weak in isolation, models a real contribution to ligand binding affinity of a bioactive agent as a whole. Illustrated in **Figure 4A** are the amide binding sites extracted from the data of six co-crystallization experiments with elastase and known ligands. Illustrated in **Figure 4B** is a cluster of the highest affinity amide binding sites determined by simulation. Illustrated in **Figure 4C** are the amide ORFs of **Figure**

4B plus amides which are in the vicinity of the cluster but which appear in the simulation at the second highest affinity values. As illustrated, this last step of expanding the results by looking at neighboring lower affinity ORF binding sites helps to better model the results seen in co-crystallography. Specifically, the cluster results identify the site at which the majority of amide binding sites are seen in crystallography, but the expansion extends the results to another cleft in elastase where amides have been experimentally located. Additionally, the expansion identifies part of another cleft at which ligand interactions are seen (as will be illustrated in other Figures).

Thus, in a third mode of analysis, the features of ligand binding sites indicated by other modes of analysis are expanded upon by looking to less stringent simulation results in the vicinity of ORF clusters. The above illustration focused on a cluster of one type of ORF, but is applicable with clusters of many types of ORFs, where the expansions can be limited to one type of ORF or multiple types of ORFs.

The data in **Figures 4A-4C** illustrate an important concept. Both in actual ligand bindings and in the simulations, multiple effective binding locations and orientations for a given type of moiety can be found to overlap. This reflects the existence of multiple local energy minima. In real world actions, rather than low temperature averaging obtained by crystallography, binding interactions will reflect a range of such local minima.

In **Figures 5A** and **5B**, solutions obtained with co-crystals of elastase inhibitors are compared with data obtained by the methods herein described. In **Figure 5A**, the solutions for six co-crystallized inhibitors are shown, with the inhibitor molecules overlaid on each other (non-space-filling representation, with the elastase segment represented by a space-filling illustration). These inhibitors are trifluoroacetyl-l-lysyl-l-prolyl-p-isopropylanilide (crystal solution: Mattos et al., as submitted April 30, 1994), trifluoroacetyl-l-lysyl-l-leucyl-p-isopropylanilide (crystal solution: Mattos et al., as submitted June 22, 1994), trifluoroacetyl-l-phenylalanyl-p-isopropylanilide (crystal solution: Mattos et al., as submitted April 30, 1994), trifluoroacetyl-l-phenylalanyl-l-alanyl-p-trifluoromethylaninide (crystal solution: Mattos et al., as submitted February 14, 1995), trifluoroacetyl-l-valyl-l-alanyl-p-trifluoromethylaninide (crystal solution: Mattos et al., as submitted February 14, 1995) and n-(tert-butoxycarbonyl-alanyl-alanyl)-o-(p-nitrobenzoyl) hydroxylamine (crystal solution: Ding et al., as submitted July 10, 1995).

In **Figure 5B**, the solutions for approximately 10 ORFs, which are in their respective high affinity protein binding states are overlaid. Both methods identify a region which favors the binding of aromatic moieties. The simulation process achieves approximately 90% 3D geometric identity with the crystallography results.

Figures 6A and **6B** show the regions of elastase involved in binding ligands as indicated by the crystallographic data, **Figure 6A**, and as indicated by the solutions obtained from the computational method described herein, **Figure 6B**.

The simulations of the invention utilize a Monte Carlo algorithm. The form of Monte Carlo simulation useful in the present invention is described in Frenkel and Smit, "Understanding Molecular Simulation: From Algorithms to Applications,"Academic Press, New York, 1996. The simulation method can comprise:

- Locate a numeric representation of the macromolecule in a periodic cell.
- Optimize the position of the macromolecule in the cell.
- Locate all the cavities in the macromolecule, whether interior or surface cavities.
- Insert and delete the ORFs (including water) in these cavities.
- Compute the probabilities of occupation of the ORFs using a grand canonical ensemble probability density function.
- Vary the chemical potential yielding relative free energies of binding.

The methodology, grand-canonical ensemble simulation, can be introduced as follows:

Grand-Canonical Ensemble Simulations

The distinguishing feature of simulations in the grand-canonical ensemble is the change in the number of molecules (ORFs) in the system during the simulation. In other words, the sampling is not restricted to the configuration space of a given dimension but it has to be extended to a set of configuration spaces. Applicant has found, unexpectedly, that the complexity of allowing for these changing numbers of molecules and the resulting changing mass nonetheless makes the simulation computationally extremely more efficient. The change in the number of molecules corresponds to the fact that the

grand-canonical partition function Ξ is the linear combination of the corresponding canonical partition functions of a different number, N, of molecules, *Q*:

$$\Xi(T,V,\mu)=\sum_{N=}^{\infty}\frac{\exp(\mu N/kT)}{N!}Q(T,V,N) \quad (1)$$

where *T* is the absolute temperature, μ is the chemical potential, *k* is the Boltzmann constant, and Q(T,V,N) is defined by:

$$Q(T,V,N)=q^{N}\int\exp(-E(X^{N})/kT)dX^{N} \quad (2)$$

with *q* being the molecular partition function.

The sampling of the configuration space of N molecules (ORFs) has been shown to be feasible using Metropolis Monte Carlo methods where in each step of the sampling a molecule's (ORFs) position is changed by a small amount and the resulting new conformation is accepted or rejected based on the change in energy, ΔE, as a result of the change attempted. This position shifting can be thought of as effecting a "shaking" of the ORF to identify its favored positioning, and the "shaking"methodology, which can be biased in the direction of the forces can be termed "forced bias Monte Carlo." When this shaking is applied, the simulation solutions reflect higher probability orientations. Accordingly:

$$P_{move}^{acc}=\min(1,\exp(-\Delta E/kT)) \quad (3)$$

Notice that the temperature (kept constant during the simulation) enters the acceptance formula as a scaling factor of the energy change.

Generalizing the canonical ensemble Metropolis method to simulations in the grand-canonical ensemble calls for steps where the number of molecules (ORFs) changes. Operationally, this requires either the deletion of an existing molecule or the 'creation', i.e., insertion of a new one. It has been shown that when the deleted molecule is chosen randomly, then the deletion attempt should be accepted with the following probability:

$$P_{del}^{acc} = \min(1, \exp(-\Delta E / kT - B)\frac{N}{V}) \quad (4)$$

where

$$B = \mu'/kT + \ln < N > \quad (5)$$

with μ' being the excess chemical potential, N the number of molecules (ORFs), $< N >$ its Boltzmann average and V the volume of the system (which is a constant during the simulation). Attempts to insert a molecule (ORF) at a random location is accepted with the following probability:

$$P_{ins}^{acc} = \min(1, \exp(-\Delta E / kT + B)\frac{V}{N+1}) \quad (6)$$

Here the effect of the chemical potential is introduced into the acceptance expression via the B parameter. The presence of the factors V and N follows from the relation between the canonical and grand-canonical partition functions: when a molecule (ORF) is taken out of the system, the integration over its coordinates (in Q_N) will yield a V factor and N is the last factor of N!. They can also be given a probabilistic interpretation: the insertion site will be chosen with probability 1/V and the molecule (ORF) to be deleted will be chosen with probability 1/N.

The simulation proceeds by alternating attempts to move, insert and delete molecules (ORFs) and accepting them with probabilities P_{move}^{acc}, P_{ins}^{acc}, P_{del}^{acc} as defined by Equations (3-5) above. After sufficiently long runs, the number of molecules (ORFs) N will fluctuate around its Boltzmann average <N>. If a given density has to be simulated then it is generally necessary to try different B values. In this regard, it is useful to note the following relationship:

$$(\frac{\partial < N >}{\partial B})T,V = < N^2 > - < N >^2 \quad (7)$$

This method has been found useful in simulating atomic fluids at moderate densities but runs into difficulties when room-temperature liquids are simulated. The difficulty stems from the fact that most insertion attempts will be at positions where there already is a molecule (e.g., from the solved protein structure) resulting in a large ΔE, and the resulting probable rejection of the attempt.

To increase the efficiency of insertion attempts, a cavity-biased insertion technique was introduced. Insertions are attempted only at sites where a cavity of suitable size already exists, thereby ensuring a non-negligible probability of acceptance. However, to ensure that the simulation thus modified still produces the required Boltzmann distribution, both the insertion and deletion acceptance probabilities have to be modified. The modified expression involves the probability of finding a cavity when there are N molecules (ORFs) present, P_N^{cav}, which follows from:

$$P_{ins}^{acc} = \min(1, \exp(-\Delta E / kT + B)\frac{P_N^{cav} V}{N+1}) \quad (8)$$

$$P_{del}^{acc} = \min(1, \exp(-\Delta E / kT - B)\frac{N}{P_N^{cav} V}) \quad (9)$$

In Equations 8 and 9, $P_N^{cav} N$ represents the volume of the regions of the system that contain cavities of suitable size. The efficiency of the cavity-biased method follows from the fact that the algorithm searching for cavities also yields P_N^{cav} without extra steps. Calculations on a variety of fluids (water, benzene), which define ORFs, have confirmed that the cavity biased method significantly increases the efficiency of insertion attempts and allows modeling of densities that proved to be impractical without this improvement.

Water binding

Aspects of the simulations used in the invention can be illustrated with calculations used to determine the strength of water binding to a synthetic polynucleotide.[1] This illustration can be described as follows:

This text illustrates how the method of *simulated annealing of chemical potential* allows bulk waters to be distinguished from bound waters, and how differentially bound waters may be distinguished from each other based on their relative chemical potentials. This is illustrated by showing that it takes more free energy to desolvate the minor groove than the major groove of a charged DNA dodecamer.

Grand canonical ensemble simulations are generally performed by placing a molecule in a periodic simulation cell, setting a parameter B, which is representative of free energy, in such a way as to achieve an experimentally determined density, sampling potential hydration positions around the molecule by inserting and deleting water molecules from the simulation cell using a technique such as cavity-bias,[2,3] and accepting or rejecting the attempt based on a Metropolis Monte Carlo[4] criteria using a grand canonical ensemble probability function.[5] The parameter B is related to the excess chemical potential μ' as follows: $B = \mu'/kT + ln\langle N\rangle$, where k is Boltzmann's constant, T is the absolute temperature, and $\langle N\rangle$ is the mean number of molecules of the ORF, which here is H_2O. In the method of *simulated annealing of chemical potential,* the simulation is started with a large initial B-value so that a higher percentage of water insertion attempts are accepted. This causes the simulation cell to be flooded with water molecules. After this grand canonical ensemble simulation at high excess chemical potential is equilibrated, subsequent simulations are carried out at successively lower B-values. This successive lowering of the B-values causes a gradual removal of the bulk water molecules from the simulation cell. As the chemical potential is further "annealed", a point is reached at which water molecules do not readily leave the cell, thereby identifying those water molecules that are strongly influenced by the DNA, the so-called "bound water molecules". As the excess chemical potential is again lowered, ultimately some of these bound waters start to leave the cell. Since chemical potential is a free energy, this *simulated annealing of chemical potential* yields a numerical estimate of the differential free energy of binding of the different bound water molecules. It must be emphasized that our utilization of the term "annealing" applies strictly to the value of

the chemical potential and that the temperature is kept constant at, for example, 298 K in all the simulations. For all simulations the DNA was held fixed, water molecules were added and deleted throughout all parts of the cell, extensive canonical Monte Carlo was performed between accepted grand canonical Monte Carlo steps, and periodic boundary conditions were used.

As an illustration of the method, a *simulated annealing of chemical potential* on a d(CGCGAATTCGCG)$_2$ was performed, starting with B = 1.0 down to –26 in 37 increments performing 2,000,000 cavity-biased grand canonical ensemble Monte Carlo steps at each B-value. The final configuration of the simulation with B = –6 , has 1120 bound water molecules. The final configuration of the simulation with B = –8, has bound 533 water molecules. The final configuration of the simulation with B = –9, has 390 bound water molecules. The final configuration of the simulation with B = –11, has 215 bound water molecules. The most salient feature of this progression is the differential hydration of the major and minor groove of the DNA. The B = –6 simulation shows the DNA essentially uniformly solvated. The B = –8 simulation clearly shows that upon lowering of the chemical potential by 2 B-units, a majority of the nonbulk extracted waters come from the major groove, while the minor groove remains almost unaffected. Annealing the chemical potential further (B = –9) still leaves the minor groove well hydrated while the major groove is almost stripped. Lowering B even further (B = –11) results in the removal of almost all water molecules from both the major and minor groove. Quantitation of the hydration of the DNA as a function of chemical potential was computed by proximity analysis[6,7] with the results shown in Table 1:

	first hydration shell				first and second hydration shell			
	minor groove		major groove		minor groove		major groove	
B	no. of waters	density	no. of waters	density	no. of waters	density	no. of waters	density
-6	7.27	0.013	13.23	0.012	21.3	0.021	41.7	0.011
-8	5.4	0.010	5.06	0.004	14.6	0.015	11.8	0.003
-9	4.08	0.007	4.36	0.004	11.5	0.011	9.7	0.003
-11	1.04	0.002	2.11	0.002	3.9	0.004	4.2	0.001

For B = –6, the first hydration shell (defined by the position of the first minimum of the radial distribution function) of the major and minor groove has a comparable density (0.012 and 0.013, respectively), while the second hydration shell of the minor groove has

twice the density of the major groove. For $B = -8$ the hydration difference becomes quite pronounced with the minor groove first and second shell hydration density being 2.5 fold and 5 fold higher than the major groove, respectively. For $B = -11$ the major and minor groove hydration density again becomes equal because at this value of the excess chemical potential both grooves are essentially stripped bare.

Illustrating the differential hydration propensities of the major and minor grooves of DNA is computationally undemanding (3 days of CPU time to run one annealing schedule and 3 days of CPU time to run one proximity analysis[7] on an SGI Power Challenge) using *simulated annealing of chemical potential* because only a coarse "cooling" schedule of the chemical potential is required. Since the chemical potential is a free energy, a very fine cooling schedule may be used to estimate quantitatively the hydration free energy difference of two different functional groups or even two different atoms of the DNA. Two atoms that desolvate at the same B-value have similar solvation free energy, or alternatively, require a finer cooling schedule to resolve the differences. It should be noted that the model system used here consisted of ionic DNA with 22 negative charges and no sodium counterions. The findings presented herein about the preferential hydration of the minor groove corresponds very well to results from X-ray crystallographic and NMR studies. Possible reasons for the stronger binding of water molecules in the minor groove may include the following: the high density of the charged rows of phosphate groups, steric constraints, and specific water—water, water—DNA interactions.

The regions where water binds tightly on a protein, are regions which are precluded from ORF binding. Thus, the remaining sites on the protein unoccupied by water are candidates for good ORF binding.

Antagonists and Agonists - Assays and Molecules

Candidate bioactive agents identified by the methods of the invention can be tested to assess their binding to the macromolecule in question. Where the macromolecules are responsible for many biological functions, including disease states, it is therefore desirable to devise screening methods to identify compounds which stimulate or which inhibit the function of the macromolecule. Accordingly, in a further aspect, the present invention provides for a method of screening compounds to identify those which stimulate or which

inhibit the function of such a macromolecule. In general, agonists or antagonists can be employed for therapeutic and prophylactic purposes for diseases. Compounds can be identified from a variety of sources, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures.

The screening methods can simply measure the binding of a candidate compound to the macromolecule, or to cells or membranes bearing the macromolecule. The macromolecule can be a variant of the macromolecule used in the simulation method, such as a fragment retaining the binding site identified in the simulation or a fusion protein used to make recombinant synthetic methods more practical. The screening method can involve competition with a labeled competitor. Further, these screening methods can test whether the candidate compound results in a signal generated by activation or inhibition of the macromolecule, using detection systems appropriate to the cells comprising the macromolecule. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed. Further, the screening methods can simply comprise the steps of mixing a candidate compound with a solution containing a macromolecule, measuring macromolecule activity in the mixture, and comparing the activity of the mixture to a standard.

The invention also provides a method of screening compounds to identify those which enhance (agonist) or block (antagonist) the action of macromolecules, including association of the macromolecule with itself or another macromolecule. The method of screening can involve high-throughput techniques. For example, to screen for agonists or antagonists, a synthetic reaction mix, a cellular compartment, such as a membrane, cell envelope or cell wall, or a preparation of any thereof, comprising macromolecule and a labeled substrate or ligand of such polypeptide is incubated in the absence or the presence of a candidate molecule that can be a agonist or antagonist. The ability of the candidate molecule to agonize or antagonize the macromolecule is reflected in decreased binding of the labeled ligand or decreased production of product from a substrate. Molecules that bind gratuitously, *i.e.*, without inducing the effects of macromolecule are most likely to be good antagonists. Molecules that bind well and, as the case can be, increase for example the rate of product production from substrate, increase signal transduction, or increase chemical channel activity are agonists. Detection of the rate or level of, as the case can be, production of

product from substrate, signal transduction, or chemical channel activity can be enhanced by using a reporter system. Reporter systems that can be useful in this regard include but are not limited to colorimetric, labeled substrate converted into product, a reporter gene that is responsive to changes in macromolecule activity, and binding assays known in the art.

References

All publications and references, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference in their entirety as if each individual publication or reference were specifically and individually indicated to be incorporated by reference herein as being fully set forth. Any patent application to which this application claims priority is also incorporated by reference herein in its entirety in the manner described above for publications and references. The cited documents incorporated by reference into this disclosure include:

[1] Guarnieri and Mezei, *J. Am. Chem. Soc.* 118: 8493-8494, 1996.
[2] Mezei, *Mol. Phys.* 61: 565-582, 1994.
[3] Resat and Mezei, *J. Am. Chem. Soc.* 116: 7451-7452, 1994.
[4] Metropolis et al., *J. Chem. Phys.* 21: 1087-1092, 1953.
[5] Tolman, R. In *The Principles of Statistical Mechanics*, Dover Press, New York, 1971.
[6] Mehrotra and Beveridge, *J. Am. Chem. Soc.* 102: 4287, 1980.
[7] The effects of different partial charges on proximity analysis are described in: Mezei, *Mol. Simul.* 1: 327-332, 1988.
[8] Calculations of volume elements can be CPU intensive. The effects of volume element calculations on proximity analysis are described in Mezei and Beveridge In *Methods in Enzymology*, Packer, Ed., Academic Press, New York, 1986, pp. 21-47.

While this invention has been described with an emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that variations in the preferred devices and methods may be used and that it is intended that the invention may be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the claims that follow.

What is claimed:

1. A method of identifying binding sites on a macromolecule comprising:

(a) for at least one organic fragment (ORF), conducting, at separate values of parameter *B*, two or more simulated annealing of chemical potential calculations using the ORF as the inserted solvent; and

(b) comparing converged solutions from step (a) to identify first locations at which the relevant ORF is strongly bound, thereby identifying candidate sites for binding ligand molecules.

2. The method of claim 1, further comprising:

(c) identifying clusters of sites that strongly bind an ORF.

3. The method of claim 2, further comprising:

(d) conducting steps (a) and (b) for each of two or more ORFs and identifying clusters where two or more distinct ORFs bind.

4. The method of claim 3, wherein a cluster that binds three or more distinct ORFs is identified.

5. The method of claim 3, further comprising reducing the binding stringency in the vicinity of a cluster to further identify elements that would contribute to the binding of a bioactive agent.

6. The method of claim 1, further comprising:

(e) conducting, at separate values a measure of chemical potential, two or more simulated annealing of chemical potential calculations using water as the inserted solvent;

(f) comparing converged solutions from step (c) to identify locations at which water is strongly bound, thereby identifying water locations which are not candidate sites for binding ligand molecules; and

(g) identifying first locations that are not water locations.

7. The method of claim 1,wherein the simulated annealing of chemical potential calculations comprise multiple steps of sampling, and wherein in a number of steps of the sampling the ORFs position is changed by a small amount and the resulting new position is accepted or rejected based on the change in energy as a result of the change attempted.

8. A method of identifying the chemical characteristics of compounds that bind a macromolecule comprising examining the functionalities and relative orientations of the ORFs found in a cluster pursuant to the binding site identifying method of claim 3.

9. A method of conducting combinatorial chemistry to identify compounds that interact with a macromolecule comprising:

(a) identifying classes of reactants that are modeled by the functionalities of the ORFs found in a cluster pursuant to the binding site identifying method of claim 3;

(b) designing a combinatorial synthetic protocol that calls for two or more synthetic procedures that react reagents of at least two of the classes identified in step (a); and

(c) conducting the combinatorial synthetic protocol to create candidate binding molecules.

10. A method of conducting a bioactive agent discovery process comprising:

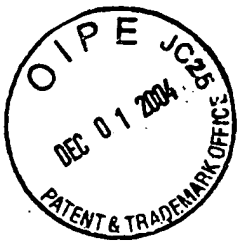
(a) from a group of established combinatorial synthetic protocols or collections of chemical compounds or pools of chemical compounds, identifying those members of the group that provide a high density of compounds that meet for a macromolecule selection criteria identified from the binding site identifying method of claim 3; and

(b) conducting binding or functional assays to identify compounds obtained from the identified collections or protocols which bind or affect the function of the macromolecule.

ABSTRACT

Provided is a method of identifying binding sites on a macromolecule comprising: **(a)** for at least one organic fragment (ORF), conducting, at separate values of parameter *B*, two or more simulated annealing of chemical potential calculations using the ORF as the inserted solvent; and **(b)** comparing converged solutions from step (a) to identify first locations at which the relevant ORF is strongly bound, thereby identifying candidate sites for binding ligand molecules.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE



In re application of:

Frank GUARNIERI

Appl. No. 09/183,267

Filed: October 30, 1998

For: **Computational Protein Probing to Identify Binding Sites**

Confirmation No.: 2934

Art Unit: 1631

Examiner: Borin, Michael L.

Atty. Docket: 1866.0010001/JMC/PEG
(*formerly* SAR-12902)

Amendment And Reply Under 37 C.F.R. § 1.111

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

In reply to the Office Action dated March 12, 2003, Applicant submits the following Amendment and Remarks. This Amendment is provided in the format prescribed in the final rule, Changes to Implement Electronic Maintenance of Official Patent Application Records, 68 Fed. Reg. 38611, 38628 (June 30, 2003) (to be codified at 37 C.F.R. pt. 1), and in the following format:

(A) Each section begins on a separate sheet;

(B) Starting on a separate sheet, amendments to the specification by presenting replacement paragraphs marked up to show changes made;

(C) Starting on a separate sheet, a complete listing of all of the claims:

- in ascending order;

- with status identifiers; and

- with markings in the currently amended claims;

(D) Starting on a separate sheet, the Remarks.

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned for under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

Amendments

In the Specification:

On page 4, on the line following the heading "Brief Description of the Drawings," please add the following paragraph:

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Please replace the paragraph beginning on page 12, line 20 with the following paragraph:

In Figures 5A and 5B, solutions obtained with co-crystals of elastase inhibitors are compared with data obtained by the methods herein described. In Figure 5A, the solutions for six co-crystallized inhibitors are shown, with the inhibitor molecules overlaid on each other (non-space-filling representation, with the elastase segment represented by a space-filling illustration). These inhibitors are trifluoroacetyl-l-lysyl-l-prolyl-p-isopropylanilide (Mattos, C. *et al., Nat. Struct. Biol. 1*:55-58 (1994); crystal solution: PDB ID: 1ELA ~~Mattos et al., as submitted April 30, 1994~~), trifluoroacetyl-l-lysyl-l-leucyl-p-isopropylanilide (Mattos, C. *et al., Nat. Struct. Biol. 1*:55-58 (1994); crystal solution: PDB ID: 1ELB ~~Mattos et al., as submitted June 22, 1994~~), trifluoroacetyl-l-phenylalanyl-p-isopropylanilide (Mattos, C. *et al., Nat. Struct. Biol. 1*:55-58 (1994); crystal solution: PDB ID: 1ELC ~~Mattos et al., as submitted April 30, 1994~~), trifluoroacetyl-l-phenylalanyl-l-alanyl-~~p-trifluoromethylanimide~~ p-trifluoromethylanilide (Mattos, C. *et al., Biochemistry 34*:3193-203 (1995); crystal solution: PDB ID: 1ELD ~~Mattos et al., as submitted February 14, 1995~~),

trifluoroacetyl-l-valyl-l-alanyl-p-trifluoromethylanilide (Mattos, C. *et al.*, *Biochemistry 34*:3193-203 (1995); crystal solution: PDB ID: 1ELE ~~Mattos et al., as submitted February 14, 1995~~) and n-(tert-butoxycarbonyl-alanyl-alanyl)-o-(p-nitrobenzoyl) hydroxylamine (Ding, X. *et al.*, *Biochemistry 34*:7749-56 (1995); crystal solution: PDB ID: 1ELF ~~Ding et al., as submitted July 10, 1995~~). In Figure 5B, the solutions for approximately 10 ORFs, which are in their respective high affinity protein binding states are overlaid. Both methods identify a region which favors the binding of aromatic moieties. The simulation process achieves approximately 90% 3D geometric identity with the crystallography results.

Please replace the paragraph beginning on page 17, line 2 with the following paragraph:

Aspects of the simulations used in the invention can be illustrated with calculations used to determine the strength of water binding to a synthetic polynucleotide (Guarnieri, F., and Mezei, M., *J. Am. Chem. Soc. 118*:8493-8494 (1996)).[[[1]]] This illustration can be described as follows:

Please replace the paragraph beginning on page 17, line 10 with the following paragraph:

Grand canonical ensemble simulations are generally performed by placing a molecule in a periodic simulation cell, setting a parameter *B*, which is representative of free energy, in such a way as to achieve an experimentally determined density, sampling potential hydration positions around the molecule by inserting and deleting water molecules from the simulation cell using a technique such as cavity-bias (Mezei, M., *Mol. Phys. 61*:565-582 (1994); Resat, H., and Mezei, M., *J. Am. Chem. Soc. 116*:7451-7452

(1994)).[[2,3]] and accepting or rejecting the attempt based on a Metropolis Monte Carlo (Metropolis, N. *et al., J. Chem. Phys. 21*:1087-1092 (1953))[[4]] criteria using a grand canonical ensemble probability function (Tolman, R., in *The Principles of Statistical Mechanics*, Dover Press, New York (1971)).[[5]] The parameter B is related to the excess chemical potential μ' as follows: $B = \mu'/kT + ln\langle N\rangle$, where k is Boltzmann's constant, T is the absolute temperature, and $\langle N\rangle$ is the mean number of molecules of the ORF, which here is H_2O. In the method of *simulated annealing of chemical potential*, the simulation is started with a large initial B-value so that a higher percentage of water insertion attempts are accepted. This causes the simulation cell to be flooded with water molecules. After this grand canonical ensemble simulation at high excess chemical potential is equilibrated, subsequent simulations are carried out at successively lower B-values. This successive lowering of the B-values causes a gradual removal of the bulk water molecules from the simulation cell. As the chemical potential is further "annealed", a point is reached at which water molecules do not readily leave the cell, thereby identifying those water molecules that are strongly influenced by the DNA, the so-called "bound water molecules". As the excess chemical potential is again lowered, ultimately some of these bound waters start to leave the cell. Since chemical potential is a free energy, this *simulated annealing of chemical potential* yields a numerical estimate of the differential free energy of binding of the different bound water molecules. It must be emphasized that our utilization of the term "annealing" applies strictly to the value of the chemical potential and that the temperature is kept constant at, for example, 298 K in all the simulations. For all simulations the DNA was held fixed, water molecules were added and deleted throughout all parts of the cell,

extensive canonical Monte Carlo was performed between accepted grand canonical Monte Carlo steps, and periodic boundary conditions were used.

Please replace the paragraph beginning on page 18, line 6 with the following paragraph:

As an illustration of the method, a *simulated annealing of chemical potential* on a d(CGCGAATTCGCG)$_2$ was performed, starting with $B = 1.0$ down to -26 in 37 increments performing 2,000,000 cavity-biased grand canonical ensemble Monte Carlo steps at each B-value. The final configuration of the simulation with B = -6, has 1120 bound water molecules. The final configuration of the simulation with B = -8, has 533 bound water molecules. The final configuration of the simulation with B = -9, has 390 bound water molecules. The final configuration of the simulation with B = -11, has 215 bound water molecules. The most salient feature of this progression is the differential hydration of the major and minor groove of the DNA. The B = -6 simulation shows the DNA essentially uniformly solvated. The B = -8 simulation clearly shows that upon lowering of the chemical potential by 2 B-units, a majority of the nonbulk extracted waters come from the major groove, while the minor groove remains almost unaffected. Annealing the chemical potential further (B = -9) still leaves the minor groove well hydrated while the major groove is almost stripped. Lowering B even further (B = -11) results in the removal of almost all water molecules from both the major and minor groove. Quantitation of the hydration of the DNA as a function of chemical potential was computed by proximity analysis (Mehrotra, P.K., and Beveridge, D.L., *J. Am. Chem. Soc. 102*:4287 (1980); Mezei, M., *Mol. Simul. 1*:327-332 (1988) (describing the effects of different partial charges on proximity analysis))[[[6,7]]] with the results shown in Table 1:

	first hydration shell				first and second hydration shell			
	minor groove		major groove		minor groove		major groove	
B	no. of waters	density	no. of waters	density	no. of waters	density	no. of waters	density
-6	7.27	0.013	13.23	0.012	21.3	0.021	41.7	0.011
-8	5.4	0.010	5.06	0.004	14.6	0.015	11.8	0.003
-9	4.08	0.007	4.36	0.004	11.5	0.011	9.7	0.003
-11	1.04	0.002	2.11	0.002	3.9	0.004	4.2	0.001

For B = -6, the first hydration shell (defined by the position of the first minimum of the radial distribution function) of the major and minor groove has a comparable density (0.012 and 0.013, respectively), while the second hydration shell of the minor groove has twice the density of the major groove. For B = -8 the hydration difference becomes quite pronounced with the minor groove first and second shell hydration density being 2.5 fold and 5 fold higher than the major groove, respectively. For B = -11 the major and minor groove hydration density again becomes equal because at this value of the excess chemical potential both grooves are essentially stripped bare.

Please replace the paragraph beginning on page 19, line 6 with the following paragraph:

Illustrating the differential hydration propensities of the major and minor grooves of DNA is computationally undemanding (3 days of CPU time to run one annealing schedule and 3 days of CPU time to run one proximity analysis (Mezei, M., and Beveridge, D.L., in *Methods in Enzymology*, Packer, ed., Academic Press, New York, pp. 21-47 (1986) (describing the effects of volume element calculations—which can be CPU intensive—on

proximity analysis))[[7]] on an SGI Power Challenge) using *simulated annealing of chemical potential* because only a coarse "cooling" schedule of the chemical potential is required. Since the chemical potential is a free energy, a very fine cooling schedule may be used to estimate quantitatively the hydration free energy difference of two different functional groups or even two different atoms of the DNA. Two atoms that desolvate at the same *B*-value have similar solvation free energy, or alternatively, require a finer cooling schedule to resolve the differences. It should be noted that the model system used here consisted of ionic DNA with 22 negative charges and no sodium counterions. The findings presented herein about the preferential hydration of the minor groove corresponds very well to results from X-ray crystallographic and NMR studies. Possible reasons for the stronger binding of water molecules in the minor groove may include the following: the high density of the charged rows of phosphate groups, steric constraints, and specific water—water, water—DNA interactions.

Please replace the paragraph beginning on page 21, line 6 with the following paragraph:

~~References~~

All publications and references, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference in their entirety as if each individual publication or reference were specifically and individually indicated to be incorporated by reference herein as being fully set forth. Any patent application to which this application claims priority is also incorporated by reference herein in its entirety in the manner described above for publications and references. ~~The cited documents incorporated by reference into this disclosure include:~~

[1]Guarnieri and Mezei, *J. Am. Chem. Soc.* 118: 8493-8494, 1996.
[2]Mezei, *Mol. Phys.* 61: 565-582, 1994.
[3]Resat and Mezei, *J. Am. Chem. Soc.* 116: 7451-7452, 1994.
[4]Metropolis et al., *J. Chem. Phys.* 21: 1087-1092, 1953.
[5]Tolman, R. In *The Principles of Statistical Mechanics*, Dover Press, New York, 1971.
[6]Mehrotra and Beveridge, *J. Am. Chem. Soc.* 102: 4287, 1980.
[7]The effects of different partial charges on proximity analysis are described in: Mezei, *Mol. Simul.* 1: 327-332, 1988.
[8]Calculations of volume elements can be CPU intensive. The effects of volume element calculations on proximity analysis are described in Mezei and Beveridge In *Methods in Enzymology*, Packer, Ed., Academic Press, New York, 1986, pp. 21-47.

In the Claims:

1. (canceled)

2. (currently amended) A computer-implemented method of analyzing a macromolecule for potential binding sites, ~~The method of claim 1, further~~ comprising:

(1) positioning an instance of a computer representation of a molecule or molecular fragment at a plurality of sampling sites of the macromolecule;

(2) selecting a value of B, wherein $B = \mu'/kT + ln\langle N\rangle$, where μ' is the excess chemical potential, k is Boltzmann's constant, T is the absolute temperature, and $\langle N\rangle$ is the mean number of molecules of the molecule or molecular fragment;

(3) repositioning the instances of the molecule or molecular fragment;

(4) accepting or rejecting each instance of the repositioned molecule or molecular fragment based on the Metropolis sampling criteria using the computed binding energy compared to the selected value of B;

(5) repeating steps (1) through (4) at a lesser value of B;

(6) outputting a list of unrejected instances of the molecule or molecular fragment,

wherein the molecule or molecular fragment of steps (1)-(6) is an organic fragment; and

(7) ~~(c) identifying clusters of sites that strongly bind an ORF~~ outputting a list of one or more clusters of sampling sites, wherein the clusters of sampling sites comprise closely located or superimposed sampling sites associated with the unrejected instances of the molecule or molecular fragment outputted in step (6).

3. (currently amended) The method ~~of~~ according to claim 2, further comprising:

(8) ~~(d) conducting steps (a) and (b) for each of two or more ORFs~~ repeating steps (1) through (6) for one or more additional molecules or molecular fragments, wherein said molecules or molecular fragments are organic fragments, wherein step (7) comprises ~~identifying clusters~~ outputting a list of one or more clusters of sampling sites, ~~where~~ wherein the clusters of sampling sites comprise closely located or superimposed sampling sites associated with unrejected instances of two or more distinct ~~ORFs~~ molecules or molecular fragments ~~bind~~ outputted in steps (6) and (8).

4. (currently amended) The method ~~of~~ according to claim 3, wherein ~~a cluster~~ ~~that binds~~ the clusters of sampling sites comprise closely located or superimposed sampling sites associated with unrejected instances of three or more distinct ~~ORFs~~ molecules or molecular fragments ~~is identified~~ outputted in steps (6) and (8).

5. (canceled)

6. (currently amended) A computer-implemented method of analyzing a macromolecule for potential binding sites, ~~The method of claim 3, further~~ comprising:

(1) positioning an instance of a computer representation of a molecule or molecular fragment at a plurality of sampling sites of the macromolecule;

(2) selecting a value of B, wherein $B = \mu'/kT + ln\langle N\rangle$, where μ' is the excess chemical potential, k is Boltzmann's constant, T is the absolute temperature, and $\langle N\rangle$ is the mean number of molecules of the molecule or molecular fragment;

(3) repositioning the instances of the molecule or molecular fragment;

(4) accepting or rejecting each instance of the repositioned molecule or molecular fragment based on the Metropolis sampling criteria using the computed binding energy compared to the selected value of B;

(5) repeating steps (1) through (4) at a lesser value of B;

wherein the molecule or molecular fragment of steps (1)-(5) is an organic fragment;

(6) outputting a list of unrejected instances of the molecule or molecular fragment;

(7) ~~(c) conducting, at separate values of a measure of chemical potential, two or more simulated annealing of chemical potential calculations using water as the inserted solvent; (f) comparing converged solutions from step (c) to identify locations at which water is strongly bound, thereby identifying water locations which are not candidate sites for binding ligand molecules; and (g) identifying first locations that are not water locations~~ repeating steps (1) through (5) wherein the molecule or molecular fragment is a water molecule, and outputting a list of the unrejected instances of the molecule or molecular fragment of step (6) that are not associated with unrejected instances of the water molecule; and

(8) outputting a list of one or more clusters of sampling sites, wherein the clusters of sampling sites comprise closely located or superimposed sampling sites associated with the unrejected instances of the molecule or molecular fragment outputted in step (7).

7-11. (canceled)

12. (currently amended) The method ~~of~~ according to claim ~~1~~ 2, wherein said ~~ORF~~ molecule or molecular fragment is selected from the group consisting of acetone, aldehyde, amide, ammonia, benzene, carboxylic acid, 1,4-diazine, ester, ether, formaldehyde, furan, imidazole, methane, methanol, phospho-acid, pyridine, pyrimidine, pyrrole, thiol[[,]] and thiophene.

13. (currently amended) The method ~~of~~ according to claim 3, further comprising:

(9) ~~identifying binding sites in the vicinity of said clusters which weakly bind ORFs~~, selecting a value *B'*, wherein *B'* is a value higher than the lowest value of *B* at which steps (1) through (4) were performed;

(10) repeating steps (1) through (5) for one or more molecules or molecular fragments, wherein the value of *B* selected in step (2) is greater than or equal to *B'*; and

(11) ~~thereby identifying clusters of binding sites~~ outputting a list of

unrejected instances of the molecules or molecular fragments that are in the vicinity of a cluster of sampling sites outputted in step (7).

14. (canceled)

15. (new) The method according to claim 6, further comprising:

(9) repeating steps (1) through (6) for one or more additional molecules or molecular fragments, wherein said molecules or molecular fragments are organic fragments, wherein step (7) comprises repeating steps (1) through (5) wherein the molecule or molecular fragment is a water molecule, and outputting a list of the unrejected instances of the molecule or molecular fragment of steps (6) and (9) that are not associated with unrejected instances of the water molecule.

16. (new) The method according to claim 15, further comprising:

(10) selecting a value B', wherein B' is a value higher than the lowest value of B at which steps (1) through (4) were performed;

(11) repeating steps (1) through (5) for one or more molecules or molecular fragments, wherein the value of B selected in step (2) is greater than or equal to B'; and

(12) outputting a list of unrejected instances of the molecules or molecular fragments that are in the vicinity of a cluster of sampling sites outputted in step (8).

17. (new) The method according to claim 2, wherein step (3) comprises using a forced bias canonical probability density function.

18. (new) The method according to claim 2, wherein step (4) comprises using a grand canonical ensemble probability density function.

In the Abstract:

Please replace the following abstract for the pending abstract:

Provided is a computer-implemented method of ~~identifying~~ analyzing a macromolecule for potential binding sites ~~on a macromolecule comprising~~ that includes: ~~(a) for at least one organic fragment (ORF), conducting, at separate values of parameter *B*, two or more simulated annealing of chemical potential calculations using the ORF as the inserted solvent;~~ positioning an instance of a computer representation of a molecule or molecular fragment at a plurality of sampling sites of the macromolecule; selecting a value of *B*, wherein $B = \mu'/kT + ln\langle N\rangle$, where μ' is the excess chemical potential, k is Boltzmann's constant, T is the absolute temperature, and $\langle N\rangle$ is the mean number of molecules of the molecule or molecular fragment; repositioning the instances of the molecule or molecular fragment; accepting or rejecting each instance of the repositioned molecule or molecular fragment based on the Metropolis sampling criteria using the computed binding energy compared to the selected value of *B*; repeating these steps at a lesser value of *B*; ~~and (b) comparing converged solutions from step (a) to identify first locations at which the relevant ORF is strongly bound, thereby identifying candidate sites for binding ligand molecules~~ outputting a list of unrejected instances of the molecule or molecular fragment, wherein the molecule or molecular fragment is an organic fragment; and outputting a list of one or more clusters of sampling sites, wherein the clusters of sampling sites include closely located or superimposed sampling sites associated with the unrejected instances of the molecule or molecular fragment.

Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 2-4, 6, 12, 13 and 15-18 are pending in the application. Claims 1, 5, 7, 8, 11 and 14 are sought to be canceled without prejudice to or disclaimer of the subject matter therein. Applicant reserves the right to file a divisional application directed to the subject matter of canceled claims 1, 5, 7, 8, 11 and 14. New claims 15-18 are sought to be added. Support for these claims can be found throughout the specification as filed, as detailed below. These claims are believed to introduce no new matter, and their entry is respectfully requested.

Claim 2 is sought to be amended. The preamble has been amended to make explicit the context for performing the claimed method. The steps for performing the simulation of the claimed method have also been made explicit. Claims 3, 4, 6 and 13 are sought to be amended similarly. Claim 12 is sought to be amended to conform its language to that of amended claim 2. Support for these changes can be found throughout the specification as filed, as detailed below. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Support for claims 2, 3 and 4 is found, *inter alia*, in original claims 2, 3 and 4, respectively; and in the specification, at page 7, lines 3-11, 14-16 and 21-24, and at page 9, lines 16-19 and page 10, line 4 through page 11, line 1, in combination with the following: page 6, lines 17-19 (definition of organic fragment); page 17, lines 16-18 (definition of *B*); page 14, line 12 through page 16, line 23 (determination of acceptance or rejection); and page 5, lines 25-26 (definition of cluster). Support for claims 6 and 15 is found, *inter alia*,

in original claim 6; and in the specification, as described for claims 2-4, above, and at page 3, lines 6-11 and page 11, lines 11-13 and 20-22. Support for claim 12 is found, *inter alia*, in original claim 12; and in the specification, at page 6, lines 17-19. Support for claims 13 and 16 is found, *inter alia*, in the specification, as described for claim 3, above; and at page 11, line 23 through page 12, line 13. Support for claim 17 is found, *inter alia*, in the specification, at page 7, lines 12-16. Support for claim 18 is found, *inter alia*, in the specification, at page 7, lines 16-19.

Based on the above amendment and the following remarks, Applicant respectfully requests that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Restriction Requirement

The Examiner withdrew unelected claim 8 from consideration and requested cancellation thereof. Solely to expedite prosecution of the above-captioned application, Applicant has canceled claim 8.

Objection to the Specification

The specification is objected to as addressing various sources of information identified only with "as submitted." Specifically, these sources are the crystal solutions of Mattos *et al.*, at page 12, lines 25, 26-27, 27-28, 29-30 and 30-31, and of Ding *et al.*, at page 12, line 32. Applicant has amended the specification to better identify these sources of this information and to correct one typographical error. Reconsideration and withdrawal of the objection to the specification is respectfully requested.

In copending divisional application no. 09/722,731, containing a specification identical to that of the above-captioned application, the Examiner requested that Applicant remove the list of references and the numerical numbering of references from the specification. The list of references, at page 21, lines 14-24, has been deleted from the captioned application as well. Applicant has deleted the numerical superscripts and inserted the corresponding references therefor. No new matter has been added. It is readily apparent that the superscript "7" on page 19, line 8 was a typographical error, and that it should have referred to the reference numbered "8". This is apparent because there is no nominal citation to reference no. 8 anywhere in the specification, the literal reading of which would render the inclusion of reference no. 8 superfluous. Thus, the replacement of the noted superscript "7" with reference no. 8 does not constitute the addition of new matter. Additionally, a grammatical error has been corrected in the paragraph beginning on page 19, line 6, which correction does not constitute the addition of new matter.

Drawings

The Examiner did not object to the drawings. However, in copending divisional application no. 09/722,731, containing drawings identical to those of the above-captioned application, the Examiner objected to the drawings under 37 C.F.R. § 1.84(g) and (l). Applicant submits herewith a petition under 37 C.F.R. § 1.84(a)(2) to accept color drawings in the captioned application. The color drawings are believed to comply with the relevant rules. The specification has been amended to comply with 37 C.F.R. § 1.84(a)(2)(iv).

Rejections under 35 U.S.C. § 112, second paragraph

Claims 1-7 and 11-14 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter that the Applicant regards as the invention. Applicant respectfully traverses. Each of the Examiner's rejections, labeled A-K, is addressed, either alone or with sufficiently similar or related rejections as to justify common treatment.

The Examiner asserts that "Parameter *B*" must be defined and that the steps involved in the "simulated annealing of chemical potential calculations" must be provided in the claims (rejection A); that it is not clear whether values of "Parameter *B*" refer to macromolecule, organic fragment, and/or both (rejection B); and that it is not clear whether two or more simulated annealings are conducted for each separate value of parameter *B* or whether one annealing is conducted at each "*B*" value (rejection C). Applicant traverses for at least the following reason.

Parameter *B* is described in the specification at, for example, page 14, line 26 through page 15, line 21. Furthermore, the specification describes Parameter *B* with reference to organic fragments. Accordingly, amended claim 2 recites Parameter *B* as $\mu'/kT + ln\langle N\rangle$, where μ' is the excess chemical potential, k is Boltzmann's constant, T is the absolute temperature, and $\langle N\rangle$ is the mean number of molecules of the molecule or molecular fragment. The amended claim further recites that steps (1)-(4) are performed at a single value of *B*; then a lesser value of *B* is chosen at which to repeat steps (1)-(4). *See* claim 2, step (5). Therefore, Applicant respectfully submits that Parameter *B* and features related thereto are not indefinite. The same arguments apply to amended claim 6, and to claims that

depend from claim 2 or claim 6. Reconsideration and withdrawal of these rejections under 35 U.S.C. § 112, second paragraph is respectfully requested.

The Examiner asserts that in claim 6 it is not clear whether steps (e)-(g) are made in addition to or in the alternative to steps (a) and (b) of claim 1 (rejection I). Applicant respectfully traverses. Amended claim 6 is written in independent claim form comprising a series of discrete steps. The claim specifies which steps are to be performed using an organic fragment, which are to be performed using water, and how the overall output is to accommodate the output of each operation. Thus, Applicant submits that there is no ambiguity about which steps the claim encompasses. Reconsideration and withdrawal of this rejection under 35 U.S.C. § 112, second paragraph is respectfully requested.

Applicant respectfully submits that the remaining rejections are overcome by the language of the amended claims. Specifically, the following rejections are directed to language that does not appear in the amended claims: D. (which "solutions", and "converged" via which steps, are used to identify "first locations"; and which one out of the plurality of ORFs is the "relevant ORF" used to identify "first locations"); E. ("strongly bound"); F. (which method steps are involved in "reducing the binding stringency"); G. (which "elements" are being identified; meaning of "contribute to the binding"); H. (which "bioactive agent" is addressed in the claim); J. (which "ligand molecules" are addressed); and K. ("strongly bound"). Reconsideration and withdrawal of these rejections under 35 U.S.C. § 112, second paragraph is respectfully requested.

Rejection under 35 U.S.C. § 112, first paragraph

Claims 2-5 and 13 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly being non-enabling for a plurality of organic fragments or for further identifications of elements contributing to the binding of biological agents. The Examiner asserts that the method steps achieving such determinations are neither illustrated in the form of working examples nor addressed in the form of explicit guidance. Applicant traverses for at least the following reasons.

The method according to amended claim 2 recites a (*i.e.*, at least one) molecule or molecular fragment. The Examiner conceded that the specification is "enabling for determining of [sic] binding sites for one ORF (or water)." Applicant submits that amended claims 3 and 4 (and new claim 15), which recite more than one organic molecule or molecular fragment, provide explicit guidance in implementing the methods claimed therein. Each of these claims specifies precisely which of the enumerated steps are to be repeated for each molecule or molecular fragment, and specifies how to adjust the output to accommodate the additional information that is generated by the additional steps. The use of the method for multiple organic fragments is described, *inter alia*, in the specification, at page 9, lines 16-19, and at page 10, line 4 through page 11, line 1. As the cited portion of the specification teaches, the same steps performed with one molecule or molecular fragment can be performed with other molecules or molecular fragments. The clusters are then identified from the combined results of the individual simulations. *See* page 9, lines 12-16, and page 5, lines 25-26.

Similarly, Applicant submits that amended claim 13 (and new claim 16) provides specific guidance in implementing the method claimed therein. This claim specifies

precisely which of the enumerated steps are to be repeated for each molecule or molecular fragment and the simulation conditions under which these steps are to be performed, and specifies the output to be generated by the additional steps. With the portions of the specification cited in the preceding paragraph, the specification at page 11, line 23 through page 12, line 13 teaches the use of the method to identify sites in the vicinity of clusters that more weakly bind molecules or molecular fragments. Specifically, the more weakly binding molecules or molecular fragments are those that appear in the simulation at less than the highest affinity value. The affinity value for the unrejected instances of a molecule or molecular fragment is larger when the simulation is run at a lower *B* value. *See, e.g.*, page 9, lines 3-11. Thus, outputting the unrejected instances of a molecule or molecular fragment for a simulation conducted at a higher value of *B* reveals the more weakly binding molecules or molecular fragments. In view of the preceding, Applicant submits that amended claims 2-4 and 13 are fully enabled. Reconsideration and withdrawal of the rejection under 35 U.S.C. § 112, first paragraph is respectfully requested.

Rejections under 35 U.S.C. § 102

Claims 6 and 14 are rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Guarnieri and Mezei, *J. Am. Chem. Soc. 118*:8493-8494 (1996) ("Guarnieri"). The Examiner asserts that Guarnieri teaches a method of identifying binding sites of water using the method of simulated annealing of chemical potential calculations with water as the inserted solvent. Further, the Examiner asserts that the calculations are carried out at several values of parameter *B*. Applicant traverses for at least the following reason.

The Guarnieri method purportedly reveals the locations of solvation of a biomolecule. However, locations of solvation, *i.e.*, locations at which water is strongly bound, are *not* candidate sites for binding ligands. *See* specification, at page 3, lines 8-11. The method of Guarnieri samples potential hydration positions around the molecule by inserting and deleting *water molecules* from the simulation cell. *See* Guarnieri, at 8493, ¶ 2, lines 4-6. The context of the reference is the crucial role that water plays in DNA and protein architecture and in many DNA and protein functions. *See id.* at ¶ 1. There is no mention of candidate sites for binding molecules or fragments other than water.

In contrast, amended claim 6 comprises a series of steps performed with a molecule or molecular fragment *other than water*. *See* claim 6, the line between steps (5) and (6). The preceding paragraph makes clear the crucial difference between water-binding sites and sites at which organic molecules or organic fragments bind. This difference is captured in step (7), for example, in which the output comprises a list of the unrejected instances of the organic molecule or organic molecular fragment that are *not* associated with unrejected instances of the water molecule. Guarnieri does not teach implementing the method of claim 6 using an organic molecule or organic fragment. Thus, Applicant respectfully submits that Guarnieri fails to teach all of the recited elements of amended claim 6. Reconsideration and withdrawal of the rejection under 35 U.S.C. § 102(b) is respectfully requested.

Rejections under 35 U.S.C. § 103

Claims 1-5, 7 and 11-13 are rejected under 35 U.S.C. § 103(a) as allegedly obvious over Guarnieri and Mezei, *J. Am. Chem. Soc. 118*:8493-94 (1996) ("Guarnieri"), in view of Resat, H. and Mezei, M., *Biophys. J. 71*:1179-90 (1996) ("Resat"), or Morgantini, P.-Y. and Kollman, P.A., *J. Am. Chem. Soc. 117*:6057-63 (1995) ("Morgantini"), or Blaskó, A. *et al.*, *J. Org. Chem. 58*:5738-47 (1993) ("Blaskó"), or Siepmann, J.I. and McDonald, I.R., *Molec. Phys. 79*:457-73 (1993) ("Siepmann"), or Koone, N. *et al.*, *J. Phys. Chem. 99*:16976-81 (1995) ("Koone"), or Gibson, K.D. and Scheraga, H.A., *J. Phys. Chem. 99*:3765-73 (1995) ("Gibson"), or Brandmeier, V. *et al.*, *Helv. Chim. Acta 77*:70-85 (1994) ("Brandmeier"), or Johnson, P.M., in *Resonance Ionization Spectroscopy*:145-50 (1990) ("Johnson"), or Basson, I. and Reynhardt, E.C., *J. Phys. D: Appl. Phys. 21*:1434-37 (1988) ("Basson"), or Ranieri, F.O. *et al.*, *Chem. Phys. 183*:187-205 (1994) ("Raineri"), or Mokrosz, J.L. *et al.*, *J. Het. Chem. 33*:1207-10 (1996) ("Mokrosz"), or Duggan, B.M and Craik, D.J., *J. Med. Chem. 39*:4007-16 (1996) ("Duggan"), or Clough, S.B. *et al.*, *Macromolecules 26*:597-600 (1993) ("Clough"), or Lunazzi, L. *et al.*, *J. Org. Chem. 62*:2263-2266 (1997) ("Lunazzi"), or Lee, T. and Jones, J.B., *J. Am. Chem. Soc. 118*:502-508 (1996) ("Lee").

The Examiner asserts that Guarnieri teaches a method of identifying binding sites of water using a method of simulated annealing of chemical potential calculations using water as the inserted solvent, but does not teach calculating binding sites for organic fragments. The Examiner further asserts that Resat, Morgantini, Blaskó, Siepmann, Koone, Gibson, Brandmeier, Johnson, Basson, Ranieri, Mokrosz, Duggan, Clough, Lunazzi and Lee collectively demonstrate the utility of applying molecular dynamics studies to various "organic fragments." Thus, the Examiner concludes it would have been obvious to one

skilled in the art at the time the invention was made to be motivated to apply the method of Guarnieri of identifying hydration sites on a macromolecule to determine binding sites for any compounds of interest. Applicant respectfully traverses for at least the following reason.

The Examiner asserts that the above references demonstrate the *utility* of applying "molecular dynamics" studies to various organic fragments. Assuming *arguendo* that the Examiner is correct, which the Applicant does not concede, such a demonstration of utility is not sufficient to establish a *prima facie* case of obviousness under 35 U.S.C. § 103. To establish a *prima facie* case of obviousness, (i) there must be some suggestion or motivation to modify the reference or to combine reference teachings, (ii) there must be a reasonable expectation of success, and (iii) the reference(s) must teach or suggest all the claim limitations. *In re Vaeck*, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991).

Guarnieri purportedly reveals the locations of solvation of a biomolecule, and it does so in the context of examining the crucial role that water plays in DNA and protein architecture. *See* Guarnieiri, at 8493, ¶ 1. The Guarnieri method samples potential hydration positions around the molecule by inserting and deleting water molecules from the simulation cell. *Id.* at ¶ 2, lines 4-6. The Examiner concedes that the reference does not teach determining binding sites for organic fragments, but asserts that motivation existed at the time of invention to apply Guarnieri's method for any compounds of interest, such as those described in the cited references. Each of these references is taken in turn.

Resat recites that "perhaps the most challenging part of the structure refinement process is the determination of the locations and the number of solvating water molecules," and it states that "the object of [the] study is to determine what are the likely locations for the solvating waters in the dCpG/proflavine crystal hydrate by using the [cavity-biased grand

canonical Monte Carlo] method." Resat, at 1180, ¶ 3, and 1182, ¶ 2. The context of these statements is Resat's assertion that solvation effects can have a significant influence on properties of biomolecules. *Id.* at 1180, ¶ 1. In Resat there is no mention of, much less any suggestion of, analyzing a macromolecule for potential binding sites.

Morgantini purportedly performs calculations using an empirical potential energy function to determine aqueous solvation free energies of ammonia, several acetamides and several amines. Morgantini, at 6057, ¶¶ 4-5. In Morgantini there is no mention of, much less any suggestion of, analyzing a macromolecule for potential binding sites.

Blaskó states that "[a] convenient way to study dynamic properties in solution is by quantifying the segmental motions in the molecule using ^{13}C spin-lattice relaxation times (T_1)." Blaskó, at 5738, ¶ 3. The stated purpose of the experiment described therein was to determine the solution structure of a phenol pendant-capped porphyrin and its iron(III) complex. *Id.* at ¶ 4. In Blaskó there is no mention of, much less any suggestion of, analyzing a macromolecule for potential binding sites.

The stated purpose of the simulations described in Siepmann was to investigate the microscopic structure of a self-assembled monolayer of hexadecyl mercaptan chemisorbed on ^{111}Au. Siepmann, at 458, ¶ 2. The configurational-bias Monte Carlo scheme was used to sample the conformations of the chains, the method of Barker and Watts was used for the rotations, and translational moves were performed in "the standard way". *Id.* at 460, ¶ 2. In Siepmann there is no mention of, much less any suggestion of, analyzing a macromolecule for potential binding sites.

The stated purpose of the experiments described in Koone was to discuss the motion of solvent molecules in small geometries, namely, to conduct experimental work in order to

provide a satisfactory theory for diffusion within the confined geometry of porous glass having average pore diameters of less than 4.0 nm. Koone, at 16976, ¶ 3. Experimental results obtained for cyclohexane and toluene were purportedly compared with molecular dynamics computer simulations data. *See id.* at 16977, ¶ 1 and 16980, ¶ 3. In Koone there is no mention of, much less any suggestion of, analyzing a macromolecule for potential binding sites.

Gibson purportedly describes the application of a potential to study the possible ways of packing benzene in a crystal by energy minimization from different starting points. Gibson, at 3765, ¶ 4. Secant-type unconstrained minimization solver with rescaling was used to minimize the energy. *Id.* at 3766, last ¶ and references therein. In Gibson there is no mention of, much less any suggestion of, analyzing a macromolecule for potential binding sites.

Brandmeier purptedly describes the synthesis and conformational study of biphenyl-containing cyclic peptides. Brandmeier, at 71, ¶ 1. A molecular dynamics study was performed, using the GROMOS-program package for the MD calculations, to obtain a survey of the conformational space that can be reached by one of the peptides of interest. *Id.* at 77, ¶ 3. Brandmeier suggests that the data discussed therein may serve as a guide for the design of synthetic biphenyl compounds with peptide conformations fixed in a β-sheet arrangement. *Id.* at 82, ¶ 2. In Brandmeier there is no mention of, much less any suggestion of, analyzing a macromolecule for potential binding sites.

Johnson purportedly examines the multiphoton ionization process to glean information about the dynamics of resonant intermediate states of carbon dioxide and pyrazine. *See* Johnson, at 145, abstract, and 150, ¶ 2. Analyzing photoelectron or field

ionization spectra, the investigators were able to follow processes ranging from photodissociation to internal energy rearrangement. *Id.* at 150, ¶ 2. In Johnson there is no mention of, much less any suggestion of, analyzing a macromolecule for potential binding sites.

Basson purportedly describes an investigation of the structure and molecular dynamics of montan wax by means of x-ray powder diffraction, differential scanning calorimetry and wide-line NMR. Basson, at 1434, ¶ 2. The "molecular dynamics" aspect of the reference consists of investigations of laboratory frame spin-lattice relaxation, spin-spin relaxation, and spin-lattice relaxation in the rotating frame, including correlations to the reorientational-translational motion of the chains. *Id.* at 1436, last ¶ through 1437, ¶ 5. In Basson there is no mention of, much less any suggestion of, analyzing a macromolecule for potential binding sites.

The stated purpose of the work described in Raineri was to generalize a previously described molecular theory of the solvation time correlation function to resolve the spatial and temporal dependence of the solvation response. Rainieri, at 188, ¶ 3. The investigators' theory of solvation dynamics is based on the premise that accurate results for the solvent response may be derived from an approximate treatment of the dynamical problem cast in terms of a *surrogate* time-dependent Hamiltonian in which the solute-solvent coupling is expressed in terms of *renormalized* interactions. *Id.* at ¶ 5. In Raineri there is no mention of, much less any suggestion of, analyzing a macromolecule for potential binding sites.

Mokrosz purportedly reports conformational studies of substituted heteroarylpyrimidines performed using ^{1}H NMR and molecular modeling methods. *See* Mokrosz, at 1207, ¶ 1. The investigators' NMR experiments suggested that 4-(2-furyl)-

2-methylaminopyrimidine exists in acetone-d6 at low temperature as an equimolar mixture of conformers and that it adopts an *s-trans* orientation. *Id.* at 1207, ¶ 2 and 1208, ¶ 1. Molecular modeling studies were performed using the PM3 and molecular dynamics approaches in order to verify the results of the conformational analysis. *Id.* at 1208, ¶ 4. In Mokrosz there is no mention of, much less any suggestion of, analyzing a macromolecule for potential binding sites.

Duggan purportedly measures NMR spin-lattice relaxation times and nuclear Overhauser enhancement factors in order to have an understanding of the internal molecular dynamics of various thyroid hormones. Duggan, at 4007, ¶ 1. The stated interest is in the internal molecular motion in bioactive species as a knowledge of the rate and amplitude of motions in solution places limits on conformations that may be expected in the bound state. *Id.* at 4008, ¶ 3. The sole mention of binding to macromolecules is as follows: "The fact that thyroxine and other thyroid hormones are able to so freely move over a moderately large region of conformational space has implications for receptor binding. . . . The conformational flexibility shown by the thyroid hormones may be required for binding." *Id.* at 4015, ¶ 1. In Duggan there is no mention of, much less any suggestion of, analyzing a macromolecule for potential binding sites.

The stated interest of Clough is in a family of substituted polyacetylenes displaying high degrees of conjugation, including extensively conjugated backbones, and possessing high charge densities, in contrast to typical substituted polyacetylenes. *See* Clough, at 597, ¶¶ 1,3. Modeling studies were undertaken to determine the structural characteristics responsible for the unique properties of these substituted polyacetylenes. *Id.* at ¶¶ 4,5. Molecular mechanics and molecular dynamics calculations yielded information relating to

charge distribution and chain conformation. *See id.* at 598, ¶ 6 through 600, ¶ 2. In Clough there is no mention of, much less any suggestion of, analyzing a macromolecule for potential binding sites.

The stated interest of Lunazzi is in the kinetic stabilization of thioaldehydes via steric hindrance, specifically via restricted rotation about the sp^2-sp^2 carbon-carbon bond in aryl or vinyl thioaldehydes. *See* Lunazzi, at 2263, ¶ 1. Thus, Lunazzi investigates the carbon-carbon rotational barrier about the Ar-CHS bond in several aryl aldehydes using NMR. *See id.*, passim. The only mention of molecular mechanics is in reference to predicting the relative dipole moments of *E* and *Z* rotamers of 3-amino-furan-2-thiocarbaldehyde. *Id.* at 2263, ¶ 5. In Lunazzi there is no mention of, much less any suggestion of, analyzing a macromolecule for potential binding sites.

The stated interest of Lee is in identifying and understanding the factors controlling enzyme substrate and inhibitor binding, particularly with respect to remote stereocontrol in catalysis. *See* Lee, at 502, ¶ 1. For the two serine proteases used in the study, the active site is *known*. *See id.* at ¶ 2. Furthermore, aldehydes are used as inhibitors because aldehydes are known to be transition state analog competitive inhibitors of serine proteases. *Id.* at 503, ¶ 2. Kinetic data was obtained, and "[m]olecular modeling was applied in order to interpret the kinetic data more completely." *Id.* at 504, ¶ 3. The modeling was used to determine the conformations of bound substrate. *See id.* at 504, ¶ 3 through 505, ¶ 1. Although the binding of an organic molecule to a macromolecule is discussed, as mentioned, the binding sites of the enzymes studied were known. In Lee there is no mention of, much less any suggestion of, analyzing a macromolecule for potential binding sites.

The Examiner's argument neglects to make any distinction between different computational methods—simply labeling them all as "molecular dynamics"—or any distinction between the claimed method, which relates to analyzing a macromolecule for potential binding sites for a molecule or molecular fragment, and the methods reported in the above references. Applicant respectfully submits that when these distinctions are properly borne in mind, a *prima facie* case of obviousness has not been established for at least the reason that there is no suggestion or motivation to modify the teachings of Guarnieri to arrive at the claimed invention, or to combine the teachings of Guarnieri with any of the cited references and subsequently modify them to arrive at the claimed invention. Reconsideration and withdrawal of the rejection under 35 U.S.C. § 103(a) is respectfully requested.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicant therefore respectfully requests that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicant believes that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Patrick E. Garrett
Attorney for Applicant
Registration No. 39,987

Date: 9/9/03

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UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO: 6,735,530 B1

DATED: May 11, 2004

INVENTORS: Frank GUARNIERI

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below.

Cover Page

Under section (56), please insert the following citations:

--Mezei, M., and Beveridge, D.L., "Structural Chemistry of Bimolecular Hydration via Computer Simulation: The Proximity Criterion," in *Methods in Enzymology*, Parker, ed., Academic Press, NY, pp. 21-47(1986).

Mezei, M., "Modified Proximity Criteria for the Analysis of the Solvation of a Polyfunctional Solute," *Mol. Simul. 1*:327-332, Gordon and Breach Science Publishers S.A. (1988).

Mezei, M., "Grand-canonical ensemble Monte Carlo study of dense liquid Lennard-Jones, soft spheres and water," *Mol. Phys. 61*:565-582, Taylor and Francis (1994).

Mehrotra, P.K., and Beveridge, D.L., "Structural Analysis of Molecular Solutions Based on Quasi-Component Distribution Functions," *J. Am. Chem. Soc. 102*:4287, American Chemical Society (1980).

Metropolis, N., *et al.*, "Equation of State Calculations by Fast Computing Machines," *J. Chem. Phys. 21*:1087-1092, American Institute of Physics (1953).

Resat, H., and Mezei, M., "Grand canonical Monte Carlo Simulation of Water Positions in Crystal Hydrates," *J. Am. Chem. Soc. 116*:7451-7452, American Chemical Society (1994).--.

Column 3

Line 55, please replace "Boactive" with --Bioactive--.

Column 4

Line 47, please replace ""ORFs" with --"ORFs"--.

MAILING ADDRESS OF SENDER:
Sterne, Kessler, Goldstein & Fox P.L.L.C.
1100 New York Avenue, N.W.
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PATENT NO. 6,735,530 B1
No. of additional copies
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UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

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DATED: May 11, 2004

INVENTORS: Frank GUARNIERI

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below.

Column 8

Line 19, please replace "prolyl-pisopropylanilide" with --prolyl-*p*-isopropylanilide--.

Column 9

Line 13, please replace "*Strict.*" with --*Struct.*--.

Line 19, please replace "n-tert-butoxycarbonyl" with --n-(tert-butoxycarbonyl--.

Column 10

Line 46 (equation 5), please replace "+lN>" with --+ln<N>--.

Line 66, please replace "IIV" with --1/V--.

Column 11

Line 32 (equation 8), please replace "$P_N^{cav}N$ " with --$P_N^{cav}V$ --.

Line 38, please replace "$P_N^{cav}N$ " with --$P_N^{cav}V$ --.

Column 13

Line 23, please replace "and fold" with --and 5 fold--.

Line 42, please replace "DNA:" with --DNA.--.

Column 14

Line 54, please replace "calorimetric" with --colorimetric--.

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Washington, DC 20005-3934

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UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO: 6,735,530 B1

DATED: May 11, 2004

INVENTORS: Frank GUARNIERI

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below.

Column 15

Line 10, please replace "represculation" with --representation--.

Line 13, please replace "R=" with --B=--.

Line 14, please replace "μ' the" with --μ' is the--.

Line 29, please replace "is organic" with --is an organic--.

Line 34, please replace "outputted step" with --outputted in step--.

Line 40, please replace "steps" with --step--.

Line 46, please replace "of recording" with --according--.

Line 46, please delete the "a" after "wherein".

Line 50, please replace "fragment" with --fragments--.

Line 53, please replace "lower" with --lowest--.

Line 54, please replace "performed," with --performed;--.

Line 63, please replace "benzeue" with --benzene--.

Line 64, please replace "formadehyde" with --formaldehyde--.

Column 16

Line 10, please delete "further".

Line 16, please replace "μ' the" with --μ' is the--.

Line 17, please replace "constant T" with --constant, T--.

Line 17, please replace "temperature;" with --temperature,".

Line 27, please replace "leaser" with --lesser--.

Line 28, please replace "molecule molecular" with --molecule or molecular--.

Line 60, please replace "list unrejected" with --list of unrejected--.

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